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THE ANALYSIS OF SUGAR MIXTURES.[1]

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THE various methods employed in the analysis of sugars may be roughly classified as follows:

(1) Optical methods.

(2) Reduction methods.

(3) Methods involving special reactions (such as yield of hydrazone, osazone, furfural, mucic acid, etc.).

In the case of a single sugar, any one of the above methods is sufficient for the determination. The percentage, for example, of arabinose in a solution may be determined by the polariscope, from the weight of reduced copper, from the weight of diphenylhydrazone, or from the quantity of furfural obtained on distillation with hydrochloric acid. In the analysis of a mixture of sugars, however, several factors must be determined before the percentage of each admits of calculation. The most important of these factors may be said to be the copper-reducing power, since it is from this that the total sum of reducing sugars is estimated. Very complete tables have been constructed by different analysts, giving the copper equivalent of the various reducing

[1] The greater part of this paper constitutes a report by the associate referee upon "Special Methods of Sugar Analysis," for the Twenty-second Convention of the Association of Official Agricultural Chemists, Washington, November, 1905. Read at the New Orleans Meeting of the American Chemical Society.

sugars, but unfortunately the early investigators in this field have so varied the conditions of their determinations, that there is a complete lack of uniformity among the many methods of procedure. Tables and methods are frequently confused even by experienced workers, and in case a mixture of sugars is present the analyst is still further perplexed, not knowing in terms of what sugar to express his results. In such case it has been the almost universal custom among chemists to express total reducing sugars as invert sugar, although this method of expression is only correct, to quote von Lippmann,[1] "when the mixture of reducing sugars consists of equal parts, dextrose and levulose; in all other cases this value cannot be accurately determined and dependent calculations are therefore rendered more or less unreliable."

During the past few years the writer has had occasion to make many separations of various sugars, as they occur in mixtures, and in all cases has adhered to the principle of first estimating the total reducing sugar as dextrose.[2] This method of procedure, however irrational it may seem in view of the difference in reducing power of the various sugars, offers nevertheless a complete solution of all difficulties, for by the use of a constant ratio established in terms of dextrose, for each sugar, the percentages of the different constituents in a mixture can be easily calculated.

The Action of Reducing Sugars upon Fehling Solution.—It has long been known that the dextrose equivalent of the copper reduced from a Fehling solution is not a constant quantity, but varies with the composition of the reagent, time and manner of boiling, and concentration of the sugar solution employed. Soxhlet[3] showed among the first, that when a reducing sugar acted upon the Fehling solution, the first portion added reduced most strongly and the succeeding portions gradually less strongly. The statement usually made that the reducing power of a sugar diminishes with its concentration is not, however, absolutely true and requires some modification. The reducing power of a sugar will remain constant for any concentration provided the same amount

[1] Chemie der Zuckerarten, 3rd Ed., 1, 898.

[2] The names dextrose and levulose are used in accordance with the usage of technical sugar chemists in America in place of the more correct *d*-glucose and δ-*l*-fructose.

[3] J. pr. Chem. [2] 21, 227.

of copper in solution is maintained throughout the experiment. In other words, the reducing power diminishes not on account of the increased concentration of the sugar, but because less copper remains in solution to be acted upon. This can be easily demonstrated by taking sugar solutions of the same concentration and varying the amount of copper in the Fehling solution.

Example.—0.1 gram of dextrose in 25 cc. solution gave 0.1964 gram copper by Allihn's method, the 30 cc. of the copper reagent containing 0.5276 gram of copper. A second experiment was made with the same sugar solution, using, however, a Fehling solution containing 0.3312 gram copper (0.5276 gram—0.1964 gram). In this case only 0.1826 gram of reduced copper was obtained. The sum of the weights of reduced copper for the two experiments is 0.3790 gram; that required for 0.2 gram of dextrose by Allihn's method is 0.3775 gram—a difference of 0.0015 gram, which is within the limits of experimental error.

The reducing action of any sugar upon the Fehling solutions may be expressed in general terms as follows: If for the first minute quantity s of a given sugar a definite amount c of copper is reduced, then for any multiple n of s the weight of copper would be nc, if the same amount of copper in the Fehling solution were always maintained. The latter condition, however, is never realized in practice, and with the continuous removal of copper from solution the value nc becomes $nc-(n-1+n-2+n-3+\ldots\ldots n-n)k$. When working with weighable quantities of sugar, this expression should be modified to $c+(n-1)d-(n-2+n-3\ldots\ldots n-n)k$, in which d is the difference between the weights of copper for the first two members of the series s and $2s$. The values of d and of the constant k are easily determined empirically and knowing these it is possible to construct tables for any of the reducing sugars.

The difference in reducing power of the various sugars upon Fehling's solution can be expressed very simply. If a definite weight d of a sugar D and a definite weight l of a sugar L reduce the same weight of copper, then the ratio d/l will be a fixed quantity. In other words, the various reducing sugars bear a constant ratio to one another for the same weight of reduced copper. This can be made more evident by an inspection of the following table, where a comparison of the reducing power of

dextrose, levulose and galactose is given. The method of Allihn[1] was followed as in all the experimental work of this article.

TABLE I.

A. Weight of levulose taken. Gram.	B. Weight of copper found. Gram.	C. Weight of dextrose corresponding to B. Gram.	D. Ratio $\frac{C}{A}$	E. Weight of galactose taken. Gram.	F. Weight of copper found. Gram.	G. Weight of dextrose corresponding to F. Gram.	H. Ratio $\frac{G}{E}$
0.0500	0.0887	0.0453	0.906	0.0500	0.0867	0.0443	0.886
0.1000	0.1789	0.0916	0.916	0.1000	0.1772	0.0906	0.906
0.1200	0.2139	0.1100	0.917	0.1200	0.2101	0.1080	0.900
0.1500	0.2653	0.1375	0.917	0.1500	0.2595	0.1344	0.896
0.1800	0.3153	0.1650	0.917	0.1800	0.3096	0.1618	0.899
0.2000	0.3479	0.1831	0.916	0.2000	0.3408	0.1792	0.896
0.2200	0.3799	0.2013	0.915	0.2200	0.3723	0.1970	0.896
0.2500	0.4267	0.2284	0.914	0.2500	0.4223	0.2259	0.904
		Average,	0.915			Average,	0.898

The figures under A and B are taken from the table of Hönig and Jesser (Z. Ver. deutschen Zuckerindustrie, 38, 1027) whose results the writer has fully confirmed.

The figures under C and G are taken from Allihn's table.

The figures under E and F are taken from laboratory results by the writer.

In a similar manner the ratio of xylose to dextrose was found to be 0.983 and of arabinose to dextrose 1.032.

By means of these dextrose ratios, determinations can be made of any reducing sugar by Allihn's method, the weight of dextrose in the table divided by the proper dextrose ratio giving the weight of sugar sought. The advantages of such a method of analysis are apparent; only one table is required for all the reducing sugars; the necessity of having many different stock solu-

[1] The method of Allihn was chosen as it is the one adopted by the Association of Official Agricultural Chemists, and is perhaps most extensively used by sugar chemists. Allihn's method has been criticized on the ground that the two minute boiling does not effect a complete reduction of the Fehling solution. This objection has really but little force; the method is purely a conventional one and if the details are carefully followed, accurate results can be secured. For very minute quantities of reducing sugars, however, the method of Allihn is not so well adapted as the methods of Kjeldahl or Pflüger which heat twenty and thirty minutes respectively. For a full discussion of these points see Lippmann: Chemie der Zuckerarten, Vol. I, pp. 583–612.

tions of Fehling solution is avoided; and all determinations are made under perfectly similar methods of procedure.

The dextrose equivalent of a mixture of reducing sugars is equal to the sum of the dextrose equivalent of the individual sugars. The statement has been made that in a mixture of sugars the reducing power of the individual sugars is somewhat modified by the other members present. The writer has subjected this statement to a thorough test and can discover no such influence.

A number of known mixtures of sugars were analyzed by Allihn's method and the total reducing sugars as dextrose compared with the calculated sum of the dextrose equivalents. The results of the experiments are given in Table II.

TABLE II.

Sugars.	Grams sugar in 25 cc. 1.	2.	3.	Total weight of sugars. Gram.	Dextrose equivalent. Calculated. Gram.	Found. Gram.	Error.
Dextrose, levulose...	0.0967	0.0904	.....	0.1871	0.1794	0.1780	+0.0014
" " ...	0.0484	0.0452	.....	0.0936	0.0898	0.0906	—0.0008
" " ...	0.0461	0.1408	.....	0.1869	0.1749	0.1755	—0.0006
" " ...	0.0231	0.0704	.....	0.0935	0.0875	0.0877	—0.0002
" " ...	0.0740	0.0198	.....	0.0938	0.0921	0.0927	—0.0006
" galactose..	0.1786	0.0585	.....	0.2371	0.2311	0.2294	+0.0017
" " ..	0.0893	0.0293	.....	0.1186	0.1156	0.1161	—0.0005
" " ..	0.0265	0.0960	.....	0.1225	0.1127	0.1132	—0.0005
Levulose, galactose..	0.0681	0.0175	.....	0.0856	0.0780	0.0764	+0.0016
" " ..	0.0155	0.1070	.....	0.1225	0.1102	0.1097	+0.0005
" arabinose	0.1853	0.0569	.....	0.2422	0.2282	0.2267	+0.0015
" "	0.0927	0.0285	.....	0.1212	0.1141	0.1131	+0.0010
Galactose, xylose ...	0.2162	0.0429	.....	0.2591	0.2361	0.2369	—0.0008
" " ...	0.1081	0.0215	.....	0.1296	0.1181	0.1183	—0.0002
Xylose, arabinose...	0.1513	0.0433	.....	0.1946	0.1934	0.1933	+0.0001
" " ...	0.0757	0.0217	.....	0.0974	0.0967	0.0981	—0.0014
" " ...	0.0495	0.1535	.....	0.2030	0.2070	0.2083	—0.0013
" " ...	0.0248	0.0768	.....	0.1016	0.1035	0.1044	—0.0009
Dextrose, arabinose, xylose.....	0.1371	0.0226	0.0609	0.2206	0.2203	0.2210	—0.0007
Dextrose, galactose, levulose.....	0.0646	0.0822	0.0967	0.2435	0.2270	0.2280	—0.0010

The weights in columns 1, 2 and 3 are given in the order of the respective sugars as named.

The calculated dextrose equivalents of the mixtures were found by multiplying the weights of each sugar by its dextrose ratio and adding together the products.

The greatest difference between the calculated values of the dextrose equivalent and those determined by experiment is 0.0017 gram, which is within the limits of experimental error. It is therefore safe to conclude from the results of Table II that the dextrose ratio of a sugar remains the same, whether it occurs alone or in the presence of other reducing sugars.

Application of Method to Analysis of Sugar Mixtures.—In the case of mixtures containing two reducing sugars the calculations are made from determinations of the reducing power and polarization.

Let x = per cent. of a given sugar A;
y = " " " " " " B;
a = dextrose ratio of sugar A;
b = " " " " " B;
R = per cent. of total reducing sugars as dextrose;
then I, $ax+by=R$.

The polarization of a mixture of sugars is equal to the sum of the polarizations of the individual sugars present. As the S. & H. type of saccharimeter is the form of polariscope most commonly used, all polarizations were made with this instrument, 26.048 grams of material being taken for analysis, and the readings made in a 200 mm. tube. The polarization (Ventzke) of any sugar is equal to its percentage multiplied by a polarization factor, found by dividing the specific rotation of the sugar by the specific rotation of sucrose (+66.5).

Let α = polarization factor of sugar A;
β = " " " " " B;
P = polarization (Ventzke) of mixture;
then II, $\alpha x+\beta y=P$.

From formulas I and II we obtain

$$x=\frac{bP-\beta R}{\alpha b-a\beta'}$$

and

$$y=\frac{R-ax}{b}.$$

The above formulas were applied to the analysis of known mixtures of the common reducing sugars, dextrose, levulose, galactose, xylose and arabinose. The sugars employed in the work were obtained as pure as possible from reliable firms and

determinations of their specific rotations showed them to be of normal purity.

In making up the various mixtures the sugars were weighed in a small stoppered flask. After adding the requisite amount of water the flask was reweighed and the percentages of each sugar in the solution calculated. After the sugars were dissolved, the solutions were allowed to stand twenty-four hours before beginning the analysis, in order to remove all possibility of error through multirotation.

Separation of Levulose and Dextrose.—

Dextrose ratio of levulose = 0.915.

Polarization factor of levulose (20° C., 10 per cent. sol.) =

$$\frac{-90.18}{+66.5} = -1.356.$$

" " " dextrose = $\frac{+52.74}{+66.5} = 0.793.$

Owing to the great susceptibility of levulose to variations in specific rotation through changes of temperature and concentration, the use of a fixed polarization factor is only possible when the analyses are made under perfectly similar conditions. The value of the polarization factor of levulose for different temperatures and concentrations is given in the following table:

TABLE III.

Temp. Degrees.	Concentration. 1 per cent.	2 per cent.	3 per cent.	4 per cent.	5 per cent.	10 per cent.	25 per cent.
15	—1.384	—1.385	—1.387	—1.389	—1.390	—1.398	—1.422
20	—1.341	—1.343	—1.345	—1.346	—1.348	—1.356	—1.380
25	—1.299	—1.301	—1.303	—1.304	—1.306	—1.314	—1.338
30	—1.257	—1.259	—1.261	—1.262	—1.264	—1.272	—1.296

The above figures were calculated from the general formula of Jungfleisch and Grimbert, $[\alpha]_D^t = -(101.38 - 0.56t + 0.108(c - 10))$.

The variations of the polarization constant due to concentration are so small that they do not affect the accuracy of the calculations appreciably and a 10 per cent. concentration was taken as the basis. The influence of temperature, however, is so pronounced that it cannot be disregarded.

The following formulas were employed in the analysis of levulose and dextrose mixtures:

I. Per cent. levulose $(L)_{20°} = \frac{0.793R - P}{2.08}$,

II. Per cent. dextrose = R—0.915 L.

For other temperatures than 20° the value of the denominator in equation I is 2.12 (15°), 2.04 (25°), 2.00 (30°).

In the following table are given the analyses of seven mixtures containing known amounts of levulose and dextrose.

TABLE IV.

Taken.				Found.		Error.	
Levulose. Per cent.	Dextrose. Per cent.	R.	$P^{t°}$.	Levulose. Per cent.	Dextrose. Per cent.	Levulose. Per cent.	Dextrose. Per cent.
0.99	2.06	3.01	+0.35 22°	0.98	2.11	—0.01	+0.05
1.59	5.92	7.41	+2.65 23°	1.56	5.98	—0.03	+0.06
3.17	11.83	14.54	+5.30 23°	3.02	11.78	—0.15	—0.05
4.52	4.84	9.06	—2.15 22°	4.51	4.83	—0.01	—0.01
5.63	1.85	7.02	—6.00 23°	5.61	1.89	—0.02	+0.04
9.04	9.67	17.80	—4.30 22°	8.90	9.66	—0.14	—0.01
11.26	3.69	14.04	—12.00 23°	11.23	3.76	—0.03	+0.07
				Average error,		—0.06	±0.04

The percentage of invert sugar in mixtures of dextrose and levulose is easily found by combining the smaller percentage with an equal amount of the other component. The dextrose ratio of invert sugar is $\frac{1.915}{2} = 0.958$, and since 100 parts of invert sugar correspond to 95 of sucrose, the dextrose equivalent of an inverted sucrose solution gives almost exactly the amount of sucrose. One part of dextrose $= \frac{0.95}{0.958} = 0.993$ part sucrose.

Separation of Dextrose and Galactose.—

Dextrose ratio of galactose = 0.898.

Polarization factor of galactose (20°, 10 per cent. sol.) $= \frac{+80.49}{+66.5} =$ 1.21.

The specific rotation of galactose varies somewhat with temperature and concentration, the differences, however, being much less than those of levulose. The following values for the polarization factor of galactose at different temperatures and concentrations were calculated from the general formula of Meissl.[1]

TABLE V.

Temperature. Degrees.	10 per cent.	15 per cent.	20 per cent.
10	1.242	1.248	1.254
20	1.210	1.216	1.222
30	1.179	1.185	1.191

[1] J. pr. Chem. [2] 22, 97. $[\alpha]_D^t = 83.037 + 0.0785\,p - 0.209\,t$.

The concentration influence of galactose upon the polarization factor is too slight to influence the calculations appreciably; the temperature influence, however, should be regarded in case the readings are made very much above or below 20°.

The following formulas were employed in making the separations of dextrose and galactose:

I. Per cent. dextrose $(D)_{20°} = \frac{1.21\,R - 0.898\,P}{0.498}$,

II. " " galactose $= \frac{R - D}{0.898}$.

At 25° formula I would be $D = \frac{1.195\,R - 0.898\,P}{0.482}$.

In the following table are given the analyses of 4 mixtures containing known amounts of dextrose and galactose.

TABLE VI.

Taken.				Found.		Error.	
Dextrose. Per cent.	Galactose. Per cent.	R.	P[°].	Dextrose. Per cent.	Galactose. Per cent.	Dextrose. Per cent.	Galactose. Per cent.
2.12	7.68	9.06	+11.0 25°	1.97	7.89	−0.15	+0.21
4.24	15.35	18.16	+21.9 25°	4.23	15.51	−0.01	+0.16
7.15	2.34	9.29	+ 8.5 25°	7.20	2.33	+0.05	−0.01
14.29	4.68	18.35	+17.0 25°	13.82	5.04	−0.47	+0.34
				Average error,		±0.17	±0.18

The average error in the above series of experiments is nearly four times that found in the separation of levulose and dextrose. This was to be expected since, owing to the small difference in the specific rotations of dextrose and galactose, the errors of observation are doubled; in the analysis of the dextrose-levulose mixtures on the other hand the wide range in the specific rotation diminishes the experimental errors one-half (compare Formula 1 for both separations).

Separation of Levulose and Galactose.—

In making this separation the following formulas were employed:

I. Per cent. levulose $(L)_{20°} = \frac{1.21\,R - 0.898\,P}{2.324}$,

II. " " galactose $= \frac{R - 0.915\,L}{0.898}$.

The susceptibility of the specific rotations of both levulose and galactose to temperature variations necessitates a considera-

ble temperature correction if the readings are not made at 20°. This can be done by using the polarization factors given in Tables III and V. At 30°, *e. g.*, formula I would be $L = \frac{1179\,R - 0.898\,P}{2.221}$.

In the following table are given the analyses of 4 mixtures containing known amounts of levulose and galactose.

TABLE VII.

Taken.				Found.		Error.	
Levulose. Per cent.	Galactose. Per cent.	R.	$P^{t°}$.	Levulose. Per cent.	Galactose. Per cent.	Levulose. Per cent.	Galactose. Per cent.
1.24	8.56	8.78	+ 8.75 $^{28°}$	1.14	8.62	+0.10	+0.06
2.47	17.12	17.78	+17.40 $^{25°}$	2.46	17.29	—0.01	+0.17
5.44	1.40	6.11	— 5.35 $^{28°}$	5.38	1.33	—0.06	—0.07
10.89	2.80	12.31	—10.50 $^{29°}$	10.76	2.74	—0.13	—0.06
				Average error,		±0.07	±0.09

Separation of Levulose and Arabinose.—
Dextrose ratio of arabinose = 1.032.

Polarization factor of arabinose (20°) $= \frac{104.5}{66.5} = 1.571$.

The formulas employed for the separation of levulose and arabinose are the following:

I. Per cent. levulose $(L_t)_{20°} = \frac{1.571\,R - 1.032\,P}{2.836}$,

II. " " arabinose $= \frac{R - 0.915\,L}{1.032}$.

The specific rotation of arabinose varies somewhat with changes in temperature and concentration, but these variations are not great enough to seriously affect the calculations. The temperature corrections for levulose, however, must be made as usual. The denominator in equation 1 becomes 2.88 at 15°, 2.793 at 25°, and 2.75 at 30°.

In the following table are given the analyses of two mixtures containing known amounts of levulose and arabinose.

TABLE VIII.

Taken.				Found.		Error.	
Levulose. Per cent.	Arabinose. Per cent.	R.	$P^{t°}$.	Levulose. Per cent.	Arabinose. Per cent.	Levulose. Per cent.	Arabinose. Per cent.
7.41	2.28	9.05	— 6.1 $^{27°}$	7.39	2.22	—0.02	—0.06
14.82	4.55	18.14	—12.3 $^{26°}$	14.80	4.46	—0.02	—0.09
				Average error,		—0.02	—0.07

In the estimation of levulose and arabinose we have a wider range of specific rotations than with any other mixture of two sugars and a corresponding reduction in the experimental sources of error.

Separation of Xylose and Arabinose.—

Dextrose ratio of xylose = 0.983.

$$\text{Polarization factor of xylose} = \frac{+18.79}{+66.5} = 0.283.$$

The formulas employed for the separation of xylose and arabinose were the following:

I. Per cent. xylose $(X) = \frac{1.571\,R - 1.032\,P}{1.252}$,

II. " " arabinose $= \frac{R - 0.983\,X}{1.032}$.

In the following table are given the analyses of four mixtures containing known amounts of xylose and arabinose.

TABLE IX.

Taken.				Found.		Error.	
Xylose. Per cent.	Arabinose. Per cent.	R.	$P^{\frac{25°}{20°}}$.	Xylose. Per cent.	Arabinose. Per cent.	Xylose. Per cent.	Arabinose. Per cent.
1.98	6.14	8.35	+10.2	2.05	6.13	+0.07	−0.01
3.96	12.28	16.66	+20.3	4.17	12.17	+0.21	−0.11
6.05	1.73	7.85	+ 4.5	6.14	1.75	+0.09	+0.02
12.10	3.46	15.46	+ 8.8	12.14	3.42	+0.04	−0.04
				Average error,		+0.10	±0.05

The Determination of Dextrose, Levulose and Sucrose in Mixtures.—The analysis of a mixture of dextrose, levulose and sucrose is a problem which frequently confronts the sugar chemist. The polarization of a mixture of the three sugars is expressed by the formula

$$S + 0.793D - 1.356L = P_{20°}.$$

Substituting the above equation in the formulas employed for the separation of dextrose and levulose alone, we obtain, letting S equal the per cent. sucrose by Clerget:

I. Per cent. levulose $(L)_{20°} = \frac{0.793\,R + S - P}{2.08}$,

II. Per cent. dextrose[1] = R − 0.915 L.

[1] For other schemes for determining dextrose, levulose and sucrose in mixtures, see Zamaron: Bull. de l'Assoc. des Chimistes, 1902, p. 182; Buisson: Ibid. 1903, p. 499; 1904, p. 1233; Remy: Ibid. 1904, p. 1002; Stein: The Sugar Cane, 30, 206 (1898). None of these authorities, however, takes into

There are two sources of error involved in the separation by the above formulae which require some consideration. The first of these is

The Slight Reducing Action of Sucrose upon the Fehling Solution.—The reducing action of sucrose upon Fehling's solution is proportional, first, to the concentration of the sucrose and, second, to the amount of copper left unreduced. If enough reducing sugars are present to precipitate nearly all the copper from the Fehling solution the inversion of the sucrose will be very slight. This can be seen from the following series of experiments with sucrose and dextrose mixtures, in which by changing the percentages of dextrose varying amounts of copper were reduced:

TABLE X.

Expt. No.	Sucrose taken. Per cent.	Dextrose taken. Per cent.	Dextrose found. Per cent.	Error. Per cent.	Remarks.
1.....	80.00	4.00	4.82	0.82	Fehling about one-fifth reduced.
2.....	80.00	8.00	8.65	0.65	Fehling about two-fifths reduced.
3.....	80.00	16.00	16.42	0.42	Fehling about four-fifths reduced.
4.....	80.00	20.00	20.16	0.16	Fehling nearly all reduced.

Owing to the action of the greater excess of unreduced copper, five times as much sucrose was inverted in Experiment 1 as in Experiment 4, though the quantity of sucrose taken was the same in each experiment; in other words, the error due to the reducing action of sucrose is inversely proportional to the amount of dextrose present.

The following correction for this error has been found by the writer to give very concordant results: The grams of sucrose in the 25 cc of solution to be analyzed by Allihn's method are divided by the milligrams of dextrose found + 40; the quotient will give the required correction in grams to be subtracted.

The probable error involved in such a mode of correction can be seen from the following series of experiments which were made under a wide range of conditions:

account the large difference in reducing power of dextrose and levulose, so that a considerable error may result in the calculations, especially if there be a wide difference in the percentages of dextrose and levulose.

TABLE XI.

A Sucrose taken. 25 cc. Gram.	B Dextrose taken. 25 cc. Gram.	C Dextrose found. 25 cc. Gram.	D Calc. correction. Gms. sucrose÷ mgs. dextrose÷40. Gram.	E Corrected dextrose. C—D. Gram.	F Error. B—E. Gram.
0.25	0.0500	0.0523	0.0027	0.0496	+0.0004
0.25	0.1000	0.1028	0.0018	0.1010	—0.0010
0.25	0.1500	0.1518	0.0013	0.1505	—0.0005
0.25	0.2000	0.1990	0.0010	0.1980	+0.0020
0.50	0.1000	0.1045	0.0032	0.1013	—0 0013
0.50	0.1500	0.1532	0.0026	0.1506	—0.0006
0.50	0.2000	0.2032	0.0020	0.2012	—0.0012
0.50	0.2500	0.2513	0.0016	0.2497	+0.0003
1.00	0.0500	0.0603	0.0100	0.0503	—0.0003
1.00	0.1000	0.1082	0.0068	0.1014	—0.0014
1.00	0.2000	0.2053	0.0041	0.2012	—0.0012
1.00	0.2500	0.2520	0.0034	0.2486	+0.0014
2 00	0.0500	0.0666	0.0186	0.0480	+0.0020
2.00	0.1000	0.1137	0.0130	0.1007	—0.0007
2.00	0.2000	0.2075	0.0080	0.1995	+0.0005
2.00	0.2500	0.2555	0.0067	0.2488	+0.0012

The second of the errors previously referred to is the error encountered in the determination of sucrose by the Clerget method which results from

The Change in Rotation of Levulose in Neutral and Acid Solution.—The influence of this error has long been recognized; the work of Spohr, Wohl, Ost, Soxhlet, Herzfeld, Lippmann, Jungfleisch and Grimbert, Hammerschmidt, Weber, Tolman and many others, has conclusively demonstrated that the addition of hydrochloric acid increases the specific rotation of levulose. This increase naturally affects the calculation of sucrose by Clerget's formula, the effect being a plus error, which is proportional to the percentage of levulose present in the mixture and to the amount of acid employed in the inversion.

The extent of this error in practical work is shown in the following series of experiments:

TABLE XII.

Experiment No.	Levulose taken. Per cent.	Dextrose taken. Per cent.	Sucrose taken. Per cent.	Sucrose found by Clerget. Per cent.	Error. Per cent.
1	0.87	0.88	26.81	26.87	+0.06
2	1.87	1.89	14.94	15.03	+0.09
3	3.30	3.35	41.34	41.44	+0.10
4	4.10	4.14	33.59	33.77	+0.18
5	4.60	4.65	4.71	4.89	+0.18
6	7.25	7.35	21.76	21.96	+0.20
7	8.16	8.25	25.08	25.35	+0.27

The error in the determination of sucrose is thus seen to increase with the amount of levulose present. It is very evident from the above table that this error is too great to be disregarded in our commercial work; common centrifugal molasses, according to analysis made at Audubon Park, contains approximately 15 per cent. levulose and this would affect the sucrose determination by about 0.50 per cent. The correction to be deducted depends necessarily upon the concentration of the acid used for making the inversion and the dilution of the sugar solution. For 10 cc. of fuming hydrochloric acid (sp. gr. 1.18) and 100 cc. of sugar solution, the correction according to the previous table would be 0.036 per cent. for each percentage of levulose. It is interesting to note that the same value 0.036 is deduced from the results given by Lippmann,[1] showing the effect of hydrochloric acid upon the rotation of invert sugar.

In the following table the results obtained upon analyzing ten mixtures containing known amounts of sucrose, dextrose and levulose, according to the methods just described, are given.

TABLE XIII.

Taken.			Direct polarization.	t° Invert polarization.	Sucrose by Clerget.		R corrected for sucrose.	Found.		
Dextrose.	Levulose.	Sucrose.	P			R		Dextrose.	Levulose.	Sucrose (cor. for levulose)
Per ct.	Per ct.	Per ct.			Per ct.			Per ct.	Per ct.	Per ct.
1.13	0.52	6.19	+ 6.55	— 1.70 (23°)	6.23	1.69	1.65	1.20	0.49	6.22
0.52	1.11	6.24	+ 5.20	— 3.25 (18°)	6.26	1.63	1.58	0.56	1.12	6.23
2.26	1.01	12.38	+13.05	— 3.41 (23°)	12.42	3.25	3.20	2.37	0.91	12.40
1.04	2.22	12.47	+10.35	— 6.49 (1°)	12.48	3.25	3.17	1.15	2.21	12.43
5.21	9.99	15.59	+ 6.57	—14.00 (26°)	15.70	14.66	14.52	5.22	10.16	15.55
10.02	5.43	16.16	+17.06	— 4.26 (26°)	16.27	15.24	15.10	10.06	5.51	16.14
10.42	19.98	31.12	+13.14	—28.01 (26°)	31.41	28.88	28.72	10.23	20.21	30.91
20.03	10.86	32.31	+34.12	— 8.52 (24°)	32.55	29.89	29.72	19.80	10.84	32.28
5.00	5.00	90.00	+86.90	—32.01 (27°)	90.08	10.48	9.86	5.08	5.22	89.95
1.00	1.00	98.00	+97.05	—30.80	97.97	2.43	1.82	0.75	1.17	97.94
								Average error,	±0.11	±0.10 ±0.05

A number of other analyses of various sugar mixtures have

[1] Chemie der Zuckerarten, 3rd Ed., p. 922.

been made according to the principles above outlined, but the examples given are sufficient to illustrate the practicability and accuracy of the methods of separation. The work is being continued upon other mixtures, particularly those of the simpler reducing sugars with the higher saccharides maltose, lactose, and raffinose. It is needless to remark that in working with unknown mixtures the character of the different constituents must be determined by careful qualitative tests before beginning the work of analysis.

The application of some of the methods described to various problems of the sugar-cane industry, will form the subject of another paper.

[CONTRIBUTION FROM THE SUGAR EXPERIMENT STATION OF THE LOUISIANA STATE UNIVERSITY.]

THE FERMENTATION OF SUGAR-CANE PRODUCTS.[1]

BY C. A. BROWNE, JR.

Received January 1, 1906.

THAT the juice of the sugar-cane and the various products prepared therefrom are exceedingly susceptible to fermentative changes, has been known since the first beginnings of the sugar industry, for we find that the oldest writers upon the subject all make mention of the various phenomena of fermentation. The views upon the subject of fermentation, prior to the epoch-making discoveries of Pasteur, were necessarily very inexact; they are, however, exceedingly interesting in an historical way and one or two extracts from various authorities will bear quoting. The following passage from Porter's classical work upon the sugar-cane is especially characteristic: "If the fresh juice of canes is left to itself, the feculent parts are soonest decomposed. Of the first kind of feculencies one portion sinks to the bottom and the other rises to the surface, while the acid thus produced acts upon the second sort of impurities by diffusing them through the whole fluid mass. The acid which is generated at the very commencement of spontaneous decomposition holds the feculencies in most intimate combination with the fluid part. When the fermentation is well established it is continued for several weeks,

[1] Read before the New Orleans meeting of the American Chemical Society, January 1, 1906.

gradually decomposing the essential salt." The passage cited, with its obscure phraseology, sounds as if it might be taken from some ancient work on alchemy, yet the book in which it occurs was published in 1843. In discussing the practical bearings of fermentation upon the sugar industry, however, our authority treads on firmer ground, for he goes on to say: "The longer the fermentation goes forward the more difficult becomes the separation (of impurities) by heat and alkalies. If any feculencies are retained in the syrup, they very much impede the crystallization of the sugar." Wray, another well-known authority, whose book was published five years later than that of Porter, also states that "the glutinous fecula or ferment contained in the juice decomposes the sugar, converting it into alcohol." This old idea that fermentation was due to some principle inherent in the juice itself was held even beyond the time of Pasteur's investigations and faint echoes of this belief are heard even at the present time.

In the light of recent investigations upon the vegetable enzymes, we may say that this ancient conception of fermentation is in a certain measure correct, for there are certain "glutinous ferments contained in the juice" of all plants which produce spontaneously chemical changes of an exceedingly interesting character.

I. THE ACTION OF ENZYMES IN THE SUGAR-CANE.

If the green tops of a sugar-cane be well macerated, the juice expressed, and treated with an antiseptic agent, such as chloroform or thymol, it will be found that the sucrose content of the juice undergoes a gradual diminution, though no traces of microorganic life are evident, and that simultaneously with this decrease in sucrose the content of reducing sugars increases. We have here a well marked instance of the activity of the enzyme invertase which occurs almost universally throughout the vegetable kingdom, especially in the green or growing parts of plants. This presence of invertase has a very practical bearing outside of its physiological importance. The gradual falling off in sucrose content of sugar-cane which has been windrowed for any length of time is due very largely to spontaneous inversion. If the green tops of the cane are removed at the time of cutting, the diffusion of inverting enzymes into the stalk is prevented and the loss of sucrose will be much less evident.

A very marked peculiarity of sugar-cane juice, as of all vegetable juices, is the rapid darkening in color which takes place immediately after expression. This darkening is much more evident within the body of the cane, especially in the region of the eyes and growing parts, when its tissues are laid open to the air. We have here the evidence of another enzyme belonging to the class of oxydases. The intense blue coloration which the tissues and juice of plants take on with tincture of guaiac is ascribed to an oxydase; the decomposing action which plant extracts exercise upon hydrogen peroxide, discovered by Schoenbein, has been similarly explained, though Loew[1] attributes the latter phenomenon to a special enzyme, catalase, and Pozzi-Escot[2] to a new class of ferments called reductases. The juice from all parts of the sugar-cane reacts readily with both guaiac[3] and hydrogen peroxide; juices from sterilized canes, however, exhibit none of the reactions named. The catalytic action of cane juice upon hydrogen peroxide diminishes very rapidly on standing, especially in warm weather, as may be seen from the following diagram.

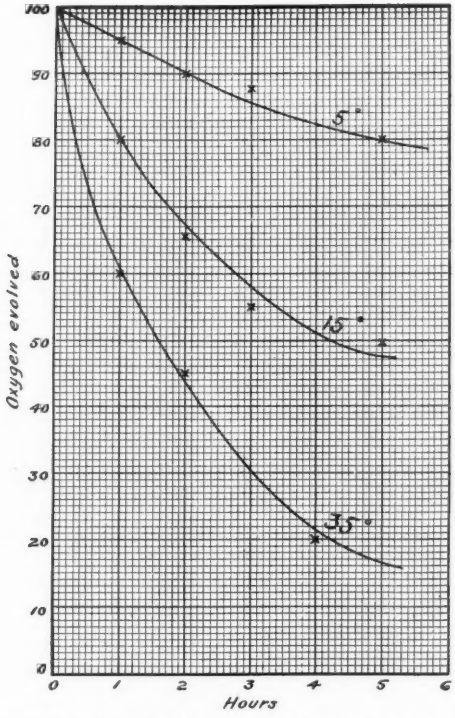
This rapid falling off in the catalytic action of cane juice on standing is probably due to some form of self-oxidation resulting from an interaction between the oxidizing and reducing enzymes.

If certain polyphenols, such as hydroquinone, or pyrogallol, are added to fresh cane juice, a rapid oxidation of these compounds is produced with an intense darkening of the juice. The

[1] Loew: "Catalase, a New Enzyme of General Occurrence," Report No. 60, U. S. Dept. Agric.

[2] Pozzi-Escot: "Reducing Enzymes," Am. Ch. J. 29, 517.

[3] Raciborski (Verslag, 1898, Proefstation voor Suikerriet in West Java, page 15) has also tested cane juice with guaiac and states that after heating above 60° C. the juice no longer gives the reaction; if, however, a little hydrogen peroxide is added the blue color disappears. This, Raciborski attributes to the presence of a substance occurring in all parts of the cane which he calls *leptomine*, and which he believes plays the part of an oxygen carrier in the respiration of all higher plants, similar to the function exercised by *haemoglobine* in the breathing of animals. We have repeated Raciborski's experiments, but have been able to confirm them only partially. Our experiments were nearly all performed upon cane which had lain several weeks in the windrow, and this may perhaps account for the differences. We found it necessary to heat the juice to from 75° to 85° C. before guaiac ceased to give the reaction. After thus destroying the *oxidase*, hydrogen peroxide was found, with some juices, to restore the color, thus indicating, perhaps, another enzyme, a *peroxidase*; with other juices no peroxidase reaction could be obtained.



latter takes on at the same time a peculiar odor, due to the formation of a quinone body, and what is more remarkable acquires a germicidal property which, in the case of the juice treated with hydroquinone, insures its preservation for weeks. Sterilized juice shows no change in color and develops no germicidal properties with any of the phenol bodies named. In connection with this oxidation of hydroquinone there is a very marked absorption of oxygen.

Two samples of cane juice (300 cc. each), one the fresh juice and the other the same juice boiled, were treated with 1 gram of hydroquinone in a closed flask. The flasks were agitated from time to time. The unboiled juice darkened rapidly, and at the end of twenty hours had turned a reddish brown; the color of the boiled juice remained unchanged. The quantity of air absorbed by each solution was determined.

	Cubic centimeters air absorbed.				
	1st day.	2d day.	3d day.	4th day.	Total.
Fresh juice	16	13	7	1	37
Boiled juice	4	5	4	3	16

The absorption in the boiled juice was probably due largely to the natural absorption of air, all dissolved gases having been previously expelled from the juice by the heating. The color of the fresh juice at the end of the experiment was almost black; the boiled juice showed no change.

The darkening of vegetable tissues on their exposure to the air, has been explained by Bertrand[1] to be due to the action of an oxidizing enzyme upon various tannin bodies, all more or less related to the polyphenols, and the query naturally arises does cane juice itself exercise any germicidal properties in connection with the natural phenomenon of darkening. The conclusion which we have reached in investigating this point is that cane juice does acquire for a time such germicidal characteristics. Counting the bacteria in the expressed juice of the cane at regular periods usually shows for several hours a uniform decrease in numbers; with juice from sterilized canes, on the other hand, the bacterial content increases from the very start.[2] The following table shows the rate of growth of bacteria in cane juices under different conditions.

TABLE I.

Experiment.	Source of juice.	Temperature.	Number of bacteria per cubic centimeter of juice. At beginning.	In 4 hours.	In 8 hours.	In 24 hours.	In 36 hours.
1	Green top	10°	2,761,920	294,336	262,080	211,800	.....
1	Green top	10°	2,681,280	241,920	231,840	187,488	.....
2	Green top	15°	106,880	96,320	98,240	.....	.....
3	Frozen cane, top	15°	260,160	274,560	320,000	960,000	.....
3	Sound cane, bottom	15°	458,880	465,600	475,840	560,000	.....
4	Steamed cane	15°	276,800	308,160	405,120	985,600	.....
4	Raw cane	15°	101,760	94,180	130,240	280,000	.....
5	Steamed cane	20°	12,480	20,160	33,600	311,040	3,840,000
5	Raw cane	20°	37,440	43,840	65,280	902,400	5,120,000

The experiments are not altogether conclusive. In Expt. 1

[1] Compt. rend. **121**, 166; **122**, 1132, 1215.

[2] In this connection it is interesting to recall the vast amount of work done by Hunziker (Bull. 197, Cornell Expt. Sta.) and others upon the germicidal action of fresh milk; it may be that certain enzymes, which we know to be always present in milk, exercise also here a toxic action.

the low temperature at which the juice and cultures were kept undoubtedly caused many organisms to succumb or remain dormant. Comparative experiments such as Nos. 4 and 5 are more satisfactory, and these show for several hours a marked retardation of bacterial growth in the raw juices, especially at the lower temperature. The tests for oxydase and catalase in cane juice became very feeble after ten or twelve hours and with this disappearance in enzymic power the number of bacteria begins to undergo a sudden increase. But it is more especially within the body of the cane itself that this germicidal action is most evident, and this we might expect not only from the colloidal and adherent character of the enzymes, which renders them resistant to expression, but from the facts of localization which will be discussed later.

If two stalks of sugar-cane, one raw and one sterilized, are punctured with a knife there will be observed at the end of a few days a marked difference in the character of the wounds. The surface of the wound in the raw cane will be discolored, but free from evidence of fermentation; the surface of the wound in the sterilized cane, on the other hand, will not be thus discolored, but will be badly infested with bacteria and moulds. When the sugar-cane is attacked by the borer or beetle, the pathway of the insect is much discolored, but we notice no inroad of organisms into the sound tissue. The living plant therefore does appear to protect itself against the invasion of microscopic parasites by forming toxic products. In case the sugar-cane is killed, and split open as sometimes happens during a freeze, this power of protection is lost (see Expt. 3, Table I). The formation of toxic products does not go on; hordes of bacteria invade the stalk, and finding no resistance start a fermentation which soon renders the cane worthless for milling.

The question may be asked if these toxic products are so deadly to micro-organic life, why do they not react unfavorably upon the cane itself? It is just here that the reducing or catalyzing enzymes perform their functions, for should the toxic oxidation products diffuse inward beyond the points of their formation, they are at once reduced and thus exert no action deeper than the exposed surface.

It will be seen that there is a great difference between working with enzymes before and after expression from the plant. As

Pfeffer remarks in his Plant Physiology, "The living cell must not be judged by reactions obtained with dead material or in the expressed juice." That there is a localization of the enzymes within the plant is rendered very evident if we apply the guaiac test to the cross-section of a cane stalk; the blue coloration is developed the strongest upon the peripheral parts, showing that the oxydases are, apparently, more localized in this region. Pozzi-Escot[1] has demonstrated that the same condition exists with other plants such as the potato; his experiments show, however, that the oxydases exist in the inner tissues as well, but that the greater localization of the reductases in these parts interferes with the guaiac reaction. Jacoby[2] goes even a step further and states that the localization of ferments exists not only in the tissues but within the individual cell. He sums up the situation very briefly in these words: "The work of the cell can be determined with absolute certainty only when we learn to separate in the test-tube the substances which are separated in the cell, and allow the individual ferments to do their work in proper sequence and in proper concentration." The difficulties of carrying out a reaction under these conditions seem almost insurmountable with our present methods of laboratory technique, yet it would be rash indeed to say that the execution of the above postulate is impossible.

II. THE DECOMPOSITION OF SUGAR-CANE PRODUCTS BY MICRO-ORGANISMS.

The number of micro-organisms which produce decomposition of cane products is almost unlimited and the chemical changes which develop, especially when several fermentations take place simultaneously, are necessarily very complex. In the brief space at our command we can only take up a few of the typical and more common fermentations.

The most common fermentation which the raw juice of the cane undergoes in Louisiana, is not the alcoholic, as might be supposed, but a fermentation designated variously as the viscous, mucilaginous or mannitic. This fermentation is anaerobic in character; in consequence, a most powerful reducing action takes place by virtue of which the juice is rapidly bleached. The liquid becomes thick and ropy, and if the culture be pure the juice will finally

[1] Am. Ch. J. 29, 530.

[2] Ergebnisse der Physiologie [1] 1, 213.

set to a perfectly solid jelly. Various organisms may produce this type of fermentation, but the best known member of this class of bacteria is the *Leuconostoc* or *Streptococcus mesenteroides*, the so-called "frog spawn" of the beet-sugar manufacturer. This fermentation was one of the first to attract the attention of investigators and the study of its products of decomposition constitutes an interesting chapter in the subject of biochemistry.

Vauquelin in 1822 caused four bottles of cane juice to be sent from Martinique to France. The samples arrived, however, in a very bad condition, the juice having changed to a thick mucilage. Vauquelin, therefore, busied himself with a study of the gummy matter into which the sugar had been changed, but the methods of organic analysis at that time were in their infancy and no definite knowledge of the gum seems to have been gained. Peligot, Kircher, Brüning and many others also occupied themselves with the problem, studying the gummy fermentation products of both cane and beet juices. Durin[1] regarded the constituent of this gum as cellulose and felt so certain of his ground that he took out a patent—a curiosity of its kind—for the "conversion of crystalline sugar into cellulose and for any use which such cellulose can find technically." It was Scheibler[2] who first established the real nature of the product; he proved the gum to be a body very similar to dextrin and named it dextran. Scheibler himself, however, fell into an error, for he regarded the gum not as a fermentation product, but as a substance occurring naturally in the plasma of the beet-cells.

Dextran was prepared from samples of clarified cane juice, which had undergone the viscous fermentation, by precipitating with 95 per cent. alcohol. The gum was filtered off and repeatedly purified by dissolving in dilute sodium hydroxide, filtering and precipitating with alcohol acidified with hydrochloric acid. After washing with alcohol and ether, the gum was dried first at 60° and then, after pulverizing, at 100°, and finally at 130°. The product thus obtained was perfectly white and contained 1.65 per cent. of ash, mostly sodium chloride.

The following analysis, calculated to ash-free substance, was obtained:

[1] "De la fermentation cellulosique du sucre de canne," Compt. rend. 83, 128 (1876).

[2] Z. Vereins deutschen Zucker-Industrie, 1869, p. 472; 1874, p. 309; 1875, p. 112.

Calculated for: $(C_6H_{10}O_5)_n$, H, 6.22 per cent.; C, 44.42 per cent.; $3(C_6H_{10}O_5).H_2O$, H, 6.40 per cent.; C, 42.83 per cent. Found: H, 6.54 per cent.; C, 42.50 per cent.

Other analysts have reported for dextran 41.45–43.61 per cent. carbon. The formula usually assigned to dextran is $(C_6H_{10}O_5)_n$, the same as that of cellulose. The writer, however, is inclined to the belief that dextran is a hydrated product of variable composition.

0.3848 gram of dextran was dissolved to 50 cc., a drop of ammonia being added to secure freedom from opalescence. The solution gave a polariscopic reading of +4.47° Ventzke in the 100 mm. tube, from which the specific rotation, $[\alpha]_D^{20} = \frac{0.3468 \times 4.47 \times 50}{0.3848} = +201.8$. The results recorded in the literature for the specific rotation of dextran vary from +195 to +230.

Ten grams of dextran were treated in the cold with 50 cc. of 90 per cent. sulphuric acid for twenty-four hours. The dark colored mixture was then poured into 500 cc. of water and heated on the steam-bath for five hours. After neutralizing with an excess of calcium carbonate, the solution was filtered and evaporated to a syrup, which was purified from gummy matter by shaking up with alcohol and ether. On filtering and evaporating a bright straw-colored syrup was obtained, which on several weeks' standing solidified to a thick mass of crystals. These were filtered off, and after recrystallizing from alcohol, using bone-black, a perfectly white sugar was obtained.

0.4514 gram of the sugar was dissolved to 10 cc. and polarized in a 100 mm. tube. Strong multirotation was noted.

TABLE II.

Time after solution.	Polariscope reading. °Ventzke.	Specific rotation. $[\alpha]_D^{20°}$.
5 minutes	+12.8	+98.3
5 hours	+ 6.9	+53.0

The constant rotation agrees with that of dextrose. No other sugar than this could be detected among the hydrolytic products of dextran.

The presence of dextran in sugar-cane products may introduce at times an error into the analytical work. It happens occasionally in Louisiana that the sugar-cane is damaged by a splitting freeze; on the occurrence of warm weather a fermentation sets in with the formation of considerable dextran within the cane. In

analyzing juices from such canes the inexperienced chemist is often puzzled because his juices polarize well, yet give him poor returns in the sugar-house. The following analyses of badly fermented cane juices will show the influence of dextran upon the polarization.

TABLE III.

No.	Degrees Brix.	Polarization.	Sucrose. Per cent.	Reducing sugars. Per cent.	Dextran. Per cent.	Apparent purity.
1	7.8	+18.0	0.0	0.15	5.90	232
2	4.8	+10.4	0.0	trace	3.35	216

The occurrence of dextran in cane syrups and molasses might lead the food chemist to suspect an adulteration of these products with commercial glucose, when in reality no such adulterant was present.

The viscous fermentation, as was stated, exerts a powerful reducing action upon the cane juice, and as a consequence of this reduction various deoxidation products are formed. The most common of these is mannite, which was very early recognized among the products of this fermentation and for this reason the name mannitic fermentation was sometimes applied. It was at first supposed that the mannite was the product of a special organism, but this is a mistake, for mannite may be formed in any fermentation of sugar where a reducing action takes place. The quantity of mannite in fermented juices will vary; juices which showed over 2 per cent. mannite were found on subsequent analysis to be nearly deficient in the same, owing to the fact that other fermentations had set in whereby the mannite was destroyed.

The separation of mannite from juices which have undergone the viscous fermentation is usually a simple matter. After removing the dextran by means of alcohol, the clear solution is filtered and evaporated, when the mannite will usually crystallize out in characteristic needles. When, however, there is a large residue of unfermented sugars, the crystallization of the mannite offers some difficulty. In such cases the writer has used a method proposed by Guignet.[1] The clarified juice is treated with a solution of copper sulphate containing a minute excess of ammonia. If mannite is present, a greenish white precipitate is formed. After some hours' standing, this is filtered off, washed with a little

[1] Ber. 22, 687.

cold water, and then, after solution in strong ammonia, decomposed with hydrogen sulphide. The copper sulphide is removed and the clear filtrate evaporated, when the mannite separates in the crystalline form. A preparation obtained from a fermented cane juice in the manner described gave on heating with concentrated hydrochloric acid and formaldehyde needles of mannite-triformacetal melting at 228°. After oxidizing with dilute nitric acid phenylhydrazine produced a white crystalline deposit of mannose-phenylhydrazone, m. p. 195°.

Among the products of the different anaerobic fermentations to which cane juice is subject, are various gaseous bodies. In cane juices clarified by the sulfitation process, especially such as have been afterwards treated with phosphoric acid, fermentation occasionally sets in and large quantities of hydrogen sulphide are evolved. The odor of this gas is usually very noticeable around fermenting press cakes. In other cases hydrogen is given off and serious accidents have been reported through the explosion of this gas, generated from juice or syrup that had been left standing in vacuum pans or effects. When fermentation of the juice and bagasse together takes place, as may occur in a diffusion battery, the cellulose of the cane fiber undergoes a decomposition, and the gas given off contains methane as well as hydrogen. Explosions of diffusion cells in sugar-beet factories from this cause have been reported not infrequently. The fermentation which takes place in bagasse piles is usually of the butyric order, as may be recognized by the peculiar rancid odor which is given off.

Reference was just made to Durin's patent for obtaining cellulose from sugar. Notwithstanding the fact that Scheibler proved Durin's cellulose to be an entirely different body, cellulose may be formed from sugar in large amounts by the activity of bacteria. A fermentation of this kind was reported by the writer[1] several years ago, and more recent investigations show that this fermentation is one of very general occurrence in Louisiana.

This fermentation, unlike the viscous, is aerobic. Large gelatinous lumps of leather-like toughness are formed in the juice. These lumps, which sometimes weigh several pounds, are stratified in appearance and are as a matter of fact made up

[1] The Louisiana Sugar Planter, 31, 305 (1903).

of an infinite number of closely compacted membranes. The substance of these membranes on boiling with alkali does not pass into solution as is the case with dextran, but shrivels up into a dense white body which gives all the reactions of cellulose, yielding a blue coloration with zinc chloride and iodine and being 99 per cent. soluble in cuprammonium. The percentage composition of the purified substance precipitated from cuprammonium agreed with that for cellulose.

Calculated for: $(C_6H_{10}O_5)_n$, H, 6.22 per cent.; C, 44.42 per cent. Found: H, 6.28 per cent.; C, 43.87 per cent.

The amount of dried membrane formed by this fermentation in a cane juice was about 13 per cent. and the amount of cellulose about 7 per cent. of the total sugar fermented. When examined under the microscope the membranous tissue is seen to consist of interwoven chains of streptococci embedded in the capsular matter, which constitutes the real substance of the membrane. Intermingled with these bacterial chains a great many yeast cells are usually visible, and it may be that we have here a case of symbiosis—such as occurs with the ginger-beer ferment, which was formerly employed quite extensively in the Southern States for making molasses beer. The cellulose-forming organism resembles very much in appearance old cultures of the *Bacterium xylinum*, which, according to A. J. Brown,[1] replaces its bacterium form in advanced stages with micrococci. The *Bacterium xylinum* also produces cellulose and the writer believes that the two organisms are identical.

Many bacteriologists and chemists are not disposed to recognize the formation of cellulose by bacteria. Emmerling,[2] for example, holds that what Brown regards as cellulose in the *Bacterium xylinum* is not cellulose at all, but chitine, and in proof of this claims to have obtained crystals of glucosamine hydrochloride on hydrolyzing the membranes with hydrochloric acid. The writer has been unable to confirm the work of Emmerling with the membranous substances obtained from cane juice. The dried membrane, after boiling with 10 per cent. caustic soda, contained only 0.2 per cent. of nitrogen and only 1 per cent. of residue

[1] "On an Acetic Ferment which Forms Cellulose," by A. J. Brown, J. Chem. Soc. 49, 432 (1886).

[2] Ber. 32, 541 (1899).

insoluble in cuprammonium, so that if chitine were present it occurred in but minute traces.

As constituents of the deposits and scums which always form in fermenting juices, syrups and molasses, we have a number of substances which, like dextran and cellulose, are to be regarded as of assimilative rather than of fermentative origin. Such, for example, is mannan; the mixed sediment of yeast cells, mycelia, etc., found in decomposed juices and syrups always contains this body. A collection of such deposits was filtered off and washed with successive portions of water, boiling alcohol and ether. The residue was then hydrolyzed with sulphuric acid in the manner already described for dextran. The solution, after neutralizing with calcium carbonate, filtering and evaporating, gave a dense white precipitate in the cold with phenylhydrazine. On recrystallizing from 60 per cent. alcohol a pure white hydrazone was obtained, soluble in boiling water and but slightly soluble in absolute alcohol or ether. The melting-point on slow heating was 188° and on rapid heating 195°. These properties agree with those of mannose-phenylhydrazone.

Another common ingredient of these fermentation products is the nitrogenous body chitine previously mentioned. We have found this substance to be a very important constituent of the scums[1] which form every year upon the surface of molasses left over in the hot-room. These scums, upon washing out the adherent molasses, constitute a brownish pulpy mass, a sample of which, air-dried, gave the following analysis:

	Per cent.
Moisture	10.00
Chitine	11.30
Protein	31.62
Fat	27.50
Ash	5.58
Undetermined (N. free)	14.00

After extracting the dried material with successive portions of ether, boiling sodium hydroxide, hot water and alcohol, about 15 per cent. of insoluble residue was obtained which analysis showed to be 90 per cent. chitine.

One gram of the purified scum residue was gently boiled in a

[1] These scums are produced by a fungus belonging to the genus *Citromyces*, for the identification of which the writer is indebted to Mrs. F. W. Patterson, of the Bureau of Plant Industry, U. S. Dept. of Agriculture.

small flask with 20 cc. of concentrated hydrochloric acid for one hour, a condenser being used to prevent evaporation. The dark colored solution was then filtered, clarified with bone-black, and evaporated until crystallization began. On cooling, a considerable quantity of white granular crystals were obtained, which were filtered off, washed with alcohol and ether, and then dried over sulphuric acid. 0.6 gram of product was obtained or 60 per cent. of the residue taken.

The crystals gave all the reactions of glucosamine hydrochloride, $C_6H_{11}(NH_2)O_5.HCl$. Heating with caustic soda yielded ammonia, and boiling with copper sulphate solution precipitated red cuprous oxide. 0.2092 gram of substance was dissolved to 10 cc. and polarized in the 100 mm. tube. The following series of readings were obtained:

TABLE IV.

Time after solution.	Polariscope reading. ° Ventzke.	Specific rotation. $[\alpha]_D^{20°}$.
5 minutes	+5.9	+97.80
30 minutes	+5.1	+84.54
1 hour	+4.8	+79.57
2 hours	+4.3	+71.28
3 hours	+4.25	+70.45
6 hours	+4.25	+70.45

Ledderhose[1] reported as the constant specific rotation of glucosamine hydrochloride prepared from lobster shells +69.54°-+70.61°.

The large amount of fat in the molasses scums (27.50 per cent.) is noteworthy, and what is more remarkable, the composition of this fat, as is shown from its physical and chemical constants, agrees very closely with that of butter fat.

	Fat from scums.	Butter fat.
Saponification number	223.1	228.5
Iodine absorption	28.17	33.35
Reichert-Meissl number	30.36	28.3
Melting-point	35°	33.2°
Melting-point, insoluble acids	41°	41.7°
Iodine number	30.53	29.5

This is the first instance so far as the writer can find of any other fat, either vegetable or animal, showing such a similarity, as regards the above constants, to butter fat. In certain respects, however, the fat differs from fresh butter fat.

[1] "Ueber Chitin und seine Spaltungsprodukte," Z. physiol. Chem. 2, 213-227 (1878-1879).

	Fat from scums.	Butter fat.
Acid number	85.2	0.50
Ether number	137.9	228.0
Mean mol. wt. soluble acids	129.7	98.1
Mean mol. wt. insoluble acids	283.2	261.0

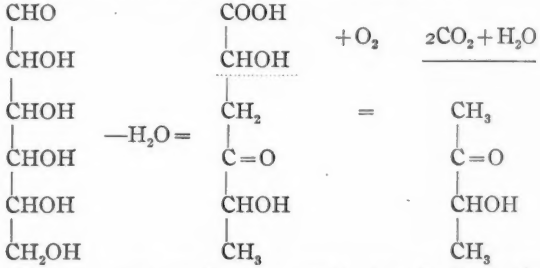
The distinguishing characteristic of the fat from the scums is the high degree of acidity and the greater preponderance of such soluble acids as caproic and caprylic. The high acid number is undoubtedly the result of hydrolysis through a lipolytic enzyme.

The physiology of fat in bacteria and other micro-organisms is a subject deserving of further investigation. The function of the fat is unquestionably that of a reserve material, and when this is utilized by the organism a series of interesting changes must result. In the hydrolysis of this fat by means of a lipase, glycerol is of course formed and this diffusing through the cell membranes enters into the fermenting liquid. Udransky[1] showed many years ago that the formation of glycerol in wines had no direct connection with the conversion of sugar into alcohol but that it was the result of metabolic activities within the yeast cell, and suggested that lecithin might be the body from which the glycerol was derived. The formation of glycerol in wines, cider, etc., by the cleavage of fatty matter within the yeast cell appears more plausible than the hypothesis of Udransky. In this cleavage some of the lower fatty acids which are liberated may unite with the alcohol forming the various fruity esters which are so marked in certain fermentations, such as that produced by *Oideum lactis*, which organism is characterized by a high content of oily matter.

It will be impossible in a paper already too long to take up other interesting fermentations of sugar-cane products, such as that produced by *Oideum lactis*, by the different varieties of *Mucor*, *Aspergillus*, and *Penicillium*, and by the various kinds of *Mycoderma*, or to discuss the character of the products formed by these various organisms. There is one fermentation product, however, which we would like to mention before concluding, as it seems to be of quite common occurrence, although not generally recognized. The presence of this compound also seems to lend support to the theory recently advanced concerning the rearrangement of the sugar molecule prior to its conversion into lactic acid or alcohol. The substance in question is dimethylketol

[1] Z. physiol. Chem. 13, 539 (1889).

or acetylmethylcarbinol, $CH_3—CO—CHOH—CH_3$. The compound was first made synthetically by von Pechmann;[1] it was discovered later by Grimbert[2] among the products produced by the fermentation of dextrose, dextrin, and mannite by *Bacillus tartricus*, and by the writer[3] among the fermentation products in cider vinegar. Pastureau[4] has also recently shown this body to be a common constituent of commercial vinegar. The writer has again found the same substance in a fermented syrup and it is his belief that this compound is always produced in small amounts whenever the alcoholic fermentation is arrested through the development of oxidizing or acid-producing bacteria. The hypothetical compound[5] into which sugar is supposed to pass before splitting up into alcohol and the oxidation of this compound to acetylmethylcarbinol can be represented as follows:



Sugar. Intermediary compound. Acetylmethylcarbinol.

It is a mistake to suppose that the fermentation of sugar-cane products is limited entirely to such dilute media as juices and syrups. Molasses is also very susceptible to fermentation, and even raw sugars during transport or in storage may undergo a gradual deterioration through the activity of yeasts and bacteria. The fermentation of such a thick menstruum as molasses, however, is confined entirely to the surface, which through the attraction of hygroscopic moisture becomes dilute enough to favor micro-organic growth. The same is true of raw sugars; the film of molasses coating the crystals undergoes a gradual fermentation

[1] Ber. 21, 2754; 22, 2214.

[2] Compt. rend. 132, 706.

[3] This Journal, 25, 31.

[4] J. pharm. chim. 21, 593 (1905).

[5] For the arguments supporting the existence of this compound see Lippmann's "Chemie der Zuckerarten," 3rd ed., p. 1890.

with the result that the underlying sucrose is slowly dissolved and inverted.

The deterioration of molasses and sugar during storage is often the cause of considerable losses in the sugar industry. A discussion of this, however, and of other economic questions, closely related to our subject, would take us far beyond the limits of our paper and must therefore be reserved for another occasion.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS.]

THE CHEMISTRY OF FLESH.

(FOURTH PAPER.)[1]

A STUDY OF THE PROTEIDS OF BEEF FLESH.[2]

BY P. F. TROWBRIDGE AND H. S. GRINDLEY.

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THE past researches upon the proteids of animal substances have been mainly devoted to the study of the proteids of blood and to the proteids of muscle freed from blood. However, the flesh of the lower animals, as it is sold for food, is a mixture of muscle and blood, each of which is a very complex substance composed of many chemical bodies in varying proportions.

Notwithstanding the large amount of valuable research which has been devoted to the chemistry of the proteids of muscle and blood, the present knowledge of these substances is very incomplete, imperfect and contradictory. This is especially true of the chemistry of muscle. The literature of this subject is at present in a state of much confusion. Hammarsten,[3] the distinguished physiological chemist, in the last edition of his text-book in considering the proteids of muscle, says: "The views of the various investigators differ so essentially and the nomenclature is so complicated that it is extremely difficult to give any correct review of the various notions. For these reasons the author is not sure whether he has understood and correctly given

[1] This Journal, **26**, 1086 (1904); **27**, 658 (1905); **28**, 25 (1906).

[2] This research was made possible by a grant from the Elizabeth Thompson Science Fund. We wish hereby gratefully to acknowledge the aid thus received in our investigations.

[3] "Text-book of Physiological Chemistry," 4th English Ed. (1904), p. 382.

the work of the different investigators. Thorough investigations on this subject are very necessary."

If the above can be said regarding the present knowledge of the proteids of muscle, it is quite evident that little is known of the chemistry of the proteids of flesh since in the first place it is a complex mixture and in the second place it has been studied but little. From the standpoint of physiological chemistry, it is highly desirable that the present very limited knowledge of the proteid substances of flesh be increased.

In a former paper[1] from this laboratory the results of the study of the solubility of the different nitrogenous constituents of flesh were given. The data thus reported were obtained by extracting flesh successively with the following reagents: Cold water, 10 per cent. sodium chloride solution, 0.15 per cent. hydrochloric acid solution, 0.15 per cent. potassium hydroxide solution and lastly with hot water. The results of the above preliminary work, together with other results obtained in later work, led to the conclusion that much additional information regarding the proteids of flesh could be obtained by extracting flesh successively with cold water, with a 10 per cent. ammonium sulphate solution, and finally with N/20 potassium hydroxide solution in the cold.

In presenting the results of the present investigation the subject may be most conveniently discussed under the following heads: A, acidity of flesh; B, proteids of flesh soluble in cold water; C, proteids of flesh insoluble in cold water but soluble in a 10 per cent. solution of ammonium sulphate; and D, proteids of flesh insoluble in cold water and in a 10 per cent. solution of ammonium sulphate but soluble in N/20 potassium hydroxide solution in the cold.

A. THE ACIDITY OF FLESH.

In order to study in detail the chemistry of the proteids of flesh, it was deemed necessary in the first place to investigate the nature of the acidity of flesh. There are at least three different classes of chemical substances which produce the acidity of flesh. They are acid phosphates, organic acids and proteids. The acid phosphates and the organic acids of flesh are mainly soluble in water while the greater portion of the proteids of flesh are insoluble in this solvent. The two chief acid phosphates

[1] This Journal, 26, 1086 (1904).

existing in the water extracts of meat are potassium dihydrogen phosphate and potassium monohydrogen phosphate. In fact these two salts are the chief mineral constituents existing in such solutions. Fractional precipitation and recrystallization of the constituents of aqueous extracts of flesh which have been made in connection with these studies have led to the conclusion that potassium dihydrogen phosphate is the acid phosphate which predominates. Potassium dihydrogen phosphate is acid to phenolphthalein and potassium monohydrogen phosphate is neutral to this indicator.

The chief organic acid occurring in flesh is lactic, which is readily soluble in water. Other soluble or insoluble organic acids exist only in very small quantities in flesh. The only indicator which at present is known to give good results with lactic acid is phenolphthalein. All other indicators are considered useless for titrating this acid.

The fact that proteids combine with bases is well-known. Osborne[1] has shown that the proteid bodies, as hitherto prepared, are in fact, definite chemical compounds of proteid substances with common mineral acids, or contain such compounds in admixture. To render such compounds neutral to phenolphthalein requires the addition of a notable quantity of an alkaline solution. The addition of the base removes the acid in combination with the proteid. If the proteids occurring in flesh are in combination with acids they will therefore probably react acid to phenolphthalein. Investigations made in this laboratory confirm this conclusion since all the proteid preparations which have so far been obtained from flesh, regardless of the method of preparation, react acid to phenolphthalein. Even the coagulated proteids separated from the aqueous extracts by continued heating in neutral solutions behave in this manner. It is thus evident that the soluble and insoluble proteids of flesh will have to be taken into account in determining the acidity of the same.

As yet methods for accurately determining the acidity due to each of these classes of substances, namely, acid phosphate, organic acids and proteids, have not been perfected. The results so far obtained indicate that it will be possible to accomplish this end in the near future.

[1] This Journal, 21, 486 (1899); 24, 39 (1902).

Acidity of the Cold Water Extracts of Flesh.—At present it is possible to determine with accuracy the total acidity of extracts of flesh when phenolphthalein is used as the indicator. Water extracts of flesh are slightly acid to litmus and azolitmin but the end reactions with these indicators in such solutions are very indefinite and unsatisfactory. The aqueous extracts are alkaline to methyl orange and neutral to cochineal; however, the former indicator would not give even a fairly sharp end reaction upon titration. None of the following indicators, rosolic acid, Congo red, lacmoid, or Bismark brown could be used on account of the very indefinite end reactions which they gave.

Preparation of the Cold Water Extracts.—Lean beef round from which all visible fat and connective tissue were removed has been used in the following work: The samples were thoroughly ground in a meat chopper and intimately mixed. For the work here reported the water extracts were prepared as follows: The finely divided meat was digested for one hour with ice water in the ratio of 1000 grams of meat to 1500 cc. of water. The resulting solution was filtered through fine cheese-cloth, the process being assisted by squeezing the cloth and its contents with the hand. The residue thus obtained was divided into smaller portions, and washed successively in series, using fresh water on number one only, until all soluble proteid was removed. Each washing was filtered through cheese-cloth as above. This operation was continued until the filtered extracts were colorless, and neutral to phenolphthalein and until they gave no reaction for proteid by the biuret test. The completely extracted residue had a grayish white color. A complete removal of the water-soluble constituents was obtained by using about 5000 cc. of water for each 1000 grams of flesh. The mixed filtrates and washings readily filtered through paper, giving a perfectly clear red filtrate.

The following table gives the results obtained in the determination of the acidity of the aqueous extracts of a number of samples of raw beef. The indicator used in the titrations was phenolphthalein.

TABLE I.—ACIDITY OF AQUEOUS EXTRACTS OF RAW BEEF FLESH.

Laboratory No.	Kind of meat.	Fresh substance. Total weight of meat taken. Grams.	Total volume of aqueous extract. cc.	Volume of N/10 alkali required for 100 cc. of extract. cc.	Volume of N/10 alkali required for 100 grams of flesh. cc.	Acidity calculated as lactic acid. Per cent.	Water-free substance. Water-free substance. Per cent.	Water-free substance. Acidity calculated as lactic acid. Per ct.
306	Beef round, lean ...	1699.000	10,000	16.54	97.34	0.88	24.31	3.62
211	Beef round, lean ...	4365.800	20,000	15.58	73.78	0.66	28.92	2.28
302	Beef round, lean ...	1585.500	11,000	12.19	84.53	0.76	26.30	2.89
1265	Beef round, lean ...	100.000	2,450	4.86	119.00	1.07	22.50	4.76
1266	Beef round, lean ...	100.000	2,500	4.22	105.60	0.95	25.28	3.76
1906	Beef round, lean ...	101.532	5,000	1.64	80.76	0.73	26.22	2.78
1916	Beef round, lean ...	99.536	5,000	2.00	100.46	0.90	26.04	3.46
	Average	.....	.....	.....	.....	0.85	.....	3.36

The total acidity of aqueous extracts of flesh varies between comparatively wide limits. The acidity of the extracts of lean beef round calculated as lactic acid and expressed in terms of the fresh substance of the meat varies from 0.66 to 1.07 per cent. This variation in the degree of acidity is probably due to the length of time, and the temperature at which the flesh has been kept from the time of the slaughtering of the animal until it is examined in the laboratory. This phase of the subject is now being studied.

Data taken from unpublished manuscript of this laboratory demonstrate the fact that the average amount of soluble inorganic phosphorus in fresh lean beef round is equal to 0.12 per cent. Assuming that all of this soluble inorganic phosphorus exists in the form of potassium dihydrogen phosphate (KH_2PO_4) there would be 0.526 per cent. of this salt. The titration of this quantity of potassium dihydrogen phosphate with a standard alkali solution using phenolphthalein as an indicator would represent a maximum acidity due to acid phosphates of 0.35 per cent. calculated as lactic acid. This calculated acidity due to acid phosphates is without doubt higher than it really should be, for it is a well-known fact that a certain, as yet unknown, proportion of the soluble inorganic phosphorus of the cold water extracts of flesh exists as dimetallic monohydrogen phosphates, *e. g.*, K_2HPO_4, which are neutral to phenolphthalein.

However, assuming for the time being that the maximum acidity due to acid phosphates may reach as much as 0.35 per

cent. calculated as lactic acid, then the average acidity of cold water extracts of flesh which is due to organic acids, proteids and other substances is at least 0.50 per cent. calculated to the same basis. How much of this latter acidity is due to each of the above-mentioned classes of substances is at present unknown, but experimental researches are now under way in this laboratory which will undoubtedly throw some light upon this problem.

Acidity of Meat Residues Insoluble in Cold Water.—The residues of meat which were left after the complete extraction with cold water were distinctly acid to litmus and to phenolphthalein, notwithstanding the fact that the last portions of the cold water extracts were entirely neutral to phenolphthalein. It has been found very difficult to determine directly the acidity of these residues because a satisfactory end reaction could not be obtained. At first the color produced by the alkali solution in the presence of phenolphthalein disappeared very readily, but towards the end of the titration the color leaves the solution slowly and much less readily. However, by suspending weighed quantities of the proteids in water, in glass-stoppered cylinders so that thorough stirring and intimate contact followed, approximately accurate results were obtainable.

Proceeding in this way a sample of completely extracted residue of beef round weighing 18.3744 grams, required 6.95 cc. of N/10 potassium hydroxide for neutralization. This is equivalent to 0.34 per cent. acidity calculated as lactic acid. The extracted residue contained 23.46 per cent. of water-free substance so that the acidity upon the water-free basis amounted to 1.45 per cent. calculated as lactic acid.

Again, a second sample of another completely extracted residue of beef round weighing 10.00 grams required 5.06 cc. of N/10 potassium hydroxide for neutralization. This is equivalent to 0.46 per cent. acidity calculated as lactic acid. In this case the extracted residue contained 30.74 per cent. of water-free substance so that the acidity as lactic acid calculated to the water-free basis equaled 1.49 per cent.

The difficulty which was experienced in obtaining a satisfactory end reaction in determining the acidity of these residues by direct titration led to an attempt to determine this quantity by indirect titration, that is, the added excess of the standard alkali was determined by titrating back with standard acid. In this

TABLE II.—ACIDITY OF INSOLUBLE MEAT RESIDUES—DETERMINED BY TREATING WITH EXCESS OF N/10 ALKALI AND THEN TITRATING BACK WITH N/10 ACID.

Laboratory No.	Kind of meat.	Fresh substance. Weight of residue. Grams.	Volume of N/10 KOH added in excess for each 100 grams of residue. cc.	Time of digestion. Hours.	Volume of N/10 HCl required to produce neutralization. cc.	Volume of N/10 KOH combined with 100 grams of residue. cc.	Acidity calculated as lactic acid. Per cent.	Water-free substance. Water-free substance. Per cent.	Water-free substance. Acidity calculated as lactic acid. Per cent.	Remarks.
203	Beef round....	18.3794	126.7	3–4	62.6	64.1	0.58	23.46	2.47	Not filtered.
301*a*	Beef round....	8.4273	1379.5	20	1065.0	314.5	2.83	23.89	11.85	Filtered.
301*b*	Beef round....	5.1906	2239.6	20	1921.6	318.0	2.86	23.89	11.97	Filtered.
307*a*	Beef round....	3.7455	372.4	3–4	233.4	139 0	1.25	....	....	Not filtered.
307*b*	Beef round....	3.0626	455.7	3–4	297.6	158.1	1.42	....	....	Not filtered.
307*c*	Beef round....	3.0702	454.6	3–4	287 8	166.8	1.50	....	....	Not filtered.
320*a*	Beef round....	10.0000	139.1	About 2	55.6	83.5	0.75	30.74	2.47	Not filtered. Clear supernatant liquid was used.
320*b*	Beef round....	10.0000	139.1	About 2	58.4	80.7	0.73	30.74	2.37	Not filtered. Clear supernatant liquid was used.
320*c*	Beef round....	10.0000	139.1	About 18	53.8	85.3	0.77	30.74	2.50	Not filtered. Clear supernatant liquid was used.
301*c*[1]	Beef round....	10.0000	2325.0	Several. Filtered over night.	1159.4	1165.6	10.49	91.96	11.47	Filtered.
301*d*[1]	Beef round....	1.3443	1729.5	Several hours.	1397.4	332.1	2.99	91.96	3.25	Not filtered.

[1] Fresh meat residues were used in all experiments except the last two, in which the samples were air-dried after washing with alcohol and ether.

way the end reaction was quite sharp and distinct, but quite unexpected results were obtained as will be seen from the analytical data given in Table II and the discussion which follows.

It is quite evident from the results given in the above table that the residues of flesh which are insoluble in cold water, when treated with excess of dilute alkali solutions behave as acids in that they neutralize alkalies. By this action a considerable proportion, in some cases practically all of the residue, is dissolved by the alkali. The insoluble residues after air-drying by the use of alcohol and ether still show the same reactivity towards dilute alkali solutions. The extent of the action varies much but seems to be dependent primarily upon the strength of the alkaline solution and upon the length of the time of action. The insoluble residues consist almost entirely of proteid matter and the neutralizing action of the same is undoubtedly due to the proteids.

The clearly filtered, dilute alkaline solutions obtained in the above manner remain clear upon neutralization and in order to cause beginning precipitation the solution must be decidedly acid. In order to show clearly the nature of these solutions of the insoluble residues there is given below in detail the results of one experiment in this connection. The moist meat residue, No. 301, which had been thoroughly washed with cold water until free from all soluble material, was distinctly acid to litmus and to phenolphthalein. The moist residue amounted to 63.00 per cent. of the original fresh meat. Duplicate samples of the same, which were dried at a temperature of 104° until constant in weight, gave as an average result 23.89 per cent. of water-free substance.

Samples of the moist residue, weighing A, 8.4273 grams and B, 5.1906 grams, were transferred to 250 cc. flasks and after moistening thoroughly with water they were each treated with 116.3 cc. of N/10 KOH solution. The samples thus treated were allowed to stand for twenty hours with frequent shaking, diluted to exactly 250 cc., thoroughly mixed and filtered through dry filters.

The residues were almost completely dissolved by this treatment. Ten cc. portions of the filtrates were titrated to neutrality to phenolphthalein with N/10 HCl, with the following average results: A, 3.59 and B, 3.99 cc. For neutralization of the entire

sample A, 89.75 cc. and B, 99.75 cc. of N/10 HCl were required. As 116.3 cc. of N/10 KOH were added to each sample to begin with, the insoluble residues by digesting, at the ordinary temperature, with the dilute alkaline solution, neutralized the following quantities of N/10 KOH solution: A, 26.55 cc. and B, 16.55 cc. These results when calculated to 100 grams of the moist residue give the following figures: A, 315.0 cc.; B, 318.8 cc.; average, 316.9 cc. of N/10 KOH solution.

The neutralized solutions containing 10 cc. portions of the original 250 cc. alkaline filtrates thus obtained were entirely clear, and required in order to cause precipitation to begin, the following quantities of N/10 HCl solution in addition to that already added to produce neutralization: A, 0.62 cc. and B, 0.41 cc. Calculated to the entire samples these data give the following figures: A, 15.50 cc. and B, 10.25 cc. These results when calculated to 100 grams of the moist residue are equal to the following quantities: A, 183.9 cc.; B, 197.5 cc.; average, 190.7 cc. of N/10 HCl.

The addition of more acid until a certain definite amount had been added increased the precipitation of proteid matter, but when the volume of acid thus added was equal to the excess of alkali previously added no further precipitation resulted. However, all of the proteid is not in this way removed from the solution and the addition of acid beyond this point causes resolution of the precipitated proteid.

After the precipitation was made as complete as possible, there was required the following amount of N/10 HCl solution to completely redissolve it: A, 1.14 cc. and B, 0.83 cc. Calculated to the entire samples these data give the following figures: A, 28.50 cc. and B, 23.25 cc. In order to effect beginning precipitation in these acid solutions it required the addition of the following amounts of N/10 KOH solution, calculated to the whole samples: A, 15.35 cc. and B, 14.65 cc. Subtracting the latter amounts from those given immediately above there is obtained for A, 13.15 cc. and for B, 8.60 cc. which represents the amount of N/10 HCl solution necessary to hold the proteids in solution. These results when calculated to 100 grams of the moist residue give the following figures: A, 156.0 cc.; B, 165.6 cc.; average 160.8 cc. of N/10 HCl. The addition of slightly more alkali

causes precipitation but the addition of alkali beyond a certain fixed amount causes the proteid to redissolve.

It should also be mentioned in this connection that fresh flesh gives reactions with dilute alkali solutions which are similar to those described above for the insoluble residues left after completely extracting flesh with cold water. That this is true can be seen from the data presented in Table III.

Further, the insoluble proteids of flesh upon digestion with N/10 hydrochloric acid solution at the ordinary temperature combine with the latter. In one experiment 10 grams of an insoluble residue which had been air-dried after washing with alcohol and ether were treated with 189.0 cc. of N/10 hydrochloric acid solution for six hours. The solution was then made up to a definite volume and filtered through a dry filter. Aliquot portions of the filtrate were titrated with N/10 potassium hydroxide solution, using phenolphthalein as an indicator. Calculating the results to the entire 10 gram portion, 117.2 cc. of N/10 potassium hydroxide solution were required to bring about neutralization. That is to say, 68.8 cc. of the N/10 hydrochloric acid solution had combined with the 10 grams of the air-dried residue of flesh. The air-dried residue contained 8.04 per cent. of water.

A discussion of the above results of the action of bases and acids upon the different preparations obtained from flesh is at present withheld until further quantitative studies which are now in progress are completed and entirely available.

B. PROTEIDS OF FLESH SOLUBLE IN COLD WATER.

Previous investigations[1] in this laboratory have demonstrated the facts: (1) that a considerable proportion of raw flesh is soluble in cold water; (2) that the cold water extract of raw flesh contains a marked quantity of the total proteid of the flesh; (3) that the extraction of flesh with cold water may be made complete; (4) that the proteid material of the cold water extract of raw flesh consists chiefly of proteids which are coagulable by heat, but there is a small amount of proteid substances which do not coagulate but are precipitated by saturating their solution with zinc sulphate, and there is also apparently a very small amount of proteid matter which is neither coagulated by heat nor precipitated by zinc sulphate but which is precipitated by tannin and salt. Recently complete chemical analyses of the cold

[1] This Journal, 26, 1086 (1904); 27, 658 (1905).

TABLE III.—ACIDITY OF FRESH MEAT DETERMINED BY TREATING WITH EXCESS OF N/10 ALKALI AND THEN TITRATING BACK WITH N/10 ACID.

		Fresh substance.						Water-free substance.		
Laboratory No.	Kind of meat.	Weight of meat taken. Grams.	Volume of N/10 KOH added in excess for each 100 grams of meat. cc.	Time of digestion. Hours.	Volume of N/10 HCl required to produce neutralization. cc.	Volume of N/10 KOH combined with 100 grams of meat. cc.	Acidity calculated as lactic acid. Per cent.	Water-free substance. Per cent.	Acidity calculated as lactic acid. Per cent.	Remarks.
300*a*	Beef round.....	9.1986	1011.0	About 18	687.5	323.5	2.91	26.30	11.06	Filtered.
300*b*	Beef round.....	8.5638	1086.0	About 18	754.7	331.3	2.98	26.30	11.33	Filtered.
305*a*	Beef round.....	5.9293	253.0	3	112.9	122.5	1 10	24.31	4.52	Not filtered.
305*b*	Beef round.....	5.0645	275.5	3	148.7	126.8	1.14	24.31	4.69	Not filtered.
305*c*	Beef round.....	5.0113	278.3	3	154.0	124.3	1.12	24 31	4.61	Not filtered.
315*a*	Beef round.....	10.0000	139.1	About 18	9.3	129.8	1.17	30.73	3.81	Not filtered. Clear, supernatant liquid used.
315*b*	Beef round.....	10.0000	139.1	About 18	Alkali 13.9	153.0	1.38	30.73	4.41	Not filtered. Clear, supernatant liquid used.
315*c*	Beef round.....	10.0000	139.1	About 18	none	139.1	1.25	30.73	4.07	Not filtered. Clear, supernatant liquid used.

water extracts of 18 samples of lean beef round have been made in connection with these researches. The average percentage of water in these samples was 73.86. The average composition[1] of water extracts of these 18 samples of raw beef round expressed in percentage of the fresh substance is as follows: Total soluble dry substance 6.46 per cent., total soluble proteid 2.51 per cent., consisting of 2.26 per cent. coagulable proteid, 0.21 per cent. albumoses and 0.02 per cent. peptones, total organic extractives 3.04 per cent., consisting of 1.23 per cent. nitrogenous and 1.80 per cent. non-nitrogenous extractives and ash 0.92 per cent. The total nitrogen in these extracts amounted to 0.795 per cent., of which 0.401 per cent. existed as proteid and 0.394 per cent. as non-proteid nitrogen.

The average amounts of total proteid existing in these eighteen samples of lean beef round equaled 18.96 per cent. It is thus evident that 13.56 per cent. of the total proteid existing in lean beef is soluble in cold water. Of this total soluble proteid 90.04 per cent. is in a form which is coagulable by heat from a neutral solution, 8.40 per cent. exists as albumoses and a very small quantity apparently exists in the form of peptones. It is not supposed that the albumoses and peptones existed as such in the original flesh. It is more than probable that the substances which are represented in the above analyses as albumoses and peptones have been formed from the original proteids of the flesh by the treatment through which the latter have passed during their extraction, separation and estimation.

Fractional Coagulation by Heat of the Proteids of Flesh Soluble in Water.—The presence of a considerable quantity of proteid substances in the cold water extracts of flesh makes it desirable to determine their character. Fractional coagulation by heat has long been used as a means of separating different proteids existing in solution. Halliburton,[2] in studying the proteids of muscle plasma, made much use of this method of fractional coagulation in order to distinguish and also to separate the different proteids of this liquid. He gives very definite temperatures at which the different fractions separate and maintains that the proteids separating at the different temperatures are not identical.

[1] Data taken from unpublished manuscript of this laboratory.

[2] J. Physiol. 8, 133 (1887); Biochemistry of Muscle and Nerve (1904).

Upon the basis of Halliburton's work it seemed best in the first place to attempt the separation of the proteids of flesh soluble in cold water by means of fractional precipitation by heat. This was considered best notwithstanding the fact that we fully realized that in the water extracts of flesh we were dealing with solutions quite different in character from those of muscle plasma especially as regards concentration, acidity, amount of other substances present and the nature of the proteids which they contain.

The method used in preparing the water extracts for this part of the work was the same as that described above (page 472). In this connection, it should be said that if the concentrated extract did not filter rapidly, it was mixed with some of the washings which gave a dilution that filtered readily through filter-paper, giving a perfectly clear solution.

A number of preliminary experiments which it is not necessary to describe here in detail led to the following information regarding the fractional coagulation of aqueous extractions of flesh.

First, during coagulation there is an increase in the acidity of the solutions.

Second, by reducing the acidity of the aqueous extracts of flesh, the coagulation of the same takes place in better form and the filtration of the solution is much facilitated.

Third, the partial neutralization of the acidity of the aqueous extracts of flesh even at the ordinary temperature causes the precipitation of some of the proteid matter which they contain.

Fourth, the complete removal of the proteid coagulating at any fixed definite temperature requires long application of heat. Further, the lower the temperature at which a coagulum is separated the longer the time of heating required to effect complete coagulation.

Fifth, there are no well defined degrees of temperature at which different coagula of aqueous extracts of flesh separate. That this statement is true is shown by the following description of several experiments. About 75 cc. of a clear water extract of fresh meat were placed in a large bath of cold water which was very gradually heated. At a temperature of 41° an opalescence was first noted. At this point, the temperature was held stationary for fifteen minutes without apparent increase in the

opalescence. The temperature of the solution was gradually increased to 43° when the opalescence became more marked. There was however no separation of a coagulum. At 44° a fine precipitate began to separate. The solution was maintained at this temperature for one hour. A considerable quantity of a nearly white precipitate settled in a finely divided condition to the bottom of the vessel, but the solution did not become clear nor did the precipitate collect as a coagulum. Again, a one liter sample of the same extract was maintained at a temperature of 43° for two hours; a decided turbidity but no distinct coagulation resulted. Upon maintaining the temperature of the solution at 43° for twelve hours a distinct coagulum was obtained. The coagulum was removed by filtration and the perfectly clear filtrate resulting was again maintained at a temperature of 43° for twelve hours. A further coagulation resulted. This second coagulation was removed by filtration and the clear filtrate again subjected to a temperature of 43° for twelve hours. This time only a very small coagulum was formed. In a third test a 100 cc. sample of the same water extract as used in the above tests was kept at a temperature of 30° for five hours. No change was noted during the first two hours; then a precipitate began gradually to collect in the bottom of the flask so that at the end of the five hours there was quite an appreciable coagulum formed.

Sixth, the coagula are obtained in loose flocculent powders, if washed thoroughly, first with water, then with strong alcohol and finally with ether and then constantly stirred while drying them free from ether. If the samples are prepared and dried in this manner, they never harden into cakes and they are very uniform in character.

Seventh, after a coagulum separating at a definite temperature is completely removed and the filtrate from the same remains perfectly clear after being heated for several hours at this fixed temperature, a slight increase of temperature of only 2° or 3° for a comparatively short time—twenty to thirty minutes—causes an additional coagulum to commence to form.

Although the results of these preliminary experiments, a summary of which is given above, indicated quite conclusively that fractional coagulation would not effect a sharp separation between the proteids of flesh soluble in water, it was determined

to investigate this subject further by separating several coagula from such solutions under known conditions and studying in detail their physical and chemical properties in order to prove definitely the differences between the proteids thus coagulated at various temperatures.

Composition of a Complete Coagulum of a Water Extract of Flesh.—A water extract was prepared from the lean of a sample of beef round taken from a fat, export steer. The method used in preparing the extract was the same as described above (page 472). A portion of the extract representing seven and one-half pounds of the meat was completely coagulated by heating it upon the water-bath until the solution had been reduced to one-fourth of the original volume.

The first coagulum which formed somewhat below 50° was nearly white, but as the temperature of the solution increased the color of the coagulum became darker while the supernatant liquid grew lighter in color, although it never became colorless.

The completely coagulated extract was filtered and thoroughly washed with boiling water. The dark brown coagulum was washed with alcohol and ether and then air-dried. This sample (laboratory No. 101) which represents the total coagulable proteid separated by heat from a water extract of lean meat was dried to constant weight at 104° and analyzed with the following average results: C, 51.71; H, 7.35; N, 15.85; S, 0.98; ash, 1.05.

The proportion of nitrogen in the different forms of combination that occur in the decomposition products produced by boiling the proteid with hydrochloric acid was determined by Hausmann's[1] method as modified by Osborne and Harris.[2]

The following average results were obtained:

TABLE IV.—PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN THE COMPLETE COAGULUM OF A WATER EXTRACT OF FLESH.

	Per cent.
Nitrogen as ammonia	1.01
Basic nitrogen	4.49
Non-basic nitrogen	10.03
Nitrogen in magnesium oxide precipitate	0.32
Nitrogen in the humin substances	0.07
Total nitrogen by summation	15.92
Total nitrogen in proteid	15.85

[1] Z. physiol. Chem. 27, 92 (1899).

[2] This Journal, 25, 323 (1904).

Methods Used in Determining the Hydrolytic Products.—As a result of a somewhat extended study of the hydrolytic products resulting from the decomposition of the proteids of flesh with boiling hydrochloric acid, it seemed desirable to modify to a certain extent the Osborne and Harris modification of the Hausmann method. The method finally adopted for the work here reported is given herewith in detail. One gram portions of the proteid were hydrolyzed by boiling them in 500 cc. Kjeldahl flasks with 100 cc. of a 20 per cent. solution of hydrochloric acid for about ten hours. The excess of acid was removed by evaporating the solution to a volume of about 3 cc. Approximately 100 cc. of water were added to the concentrated liquid, which was then filtered and the precipitate thoroughly washed and returned to the Kjeldahl flask for the determination of the amount of nitrogen present in the humin substances. The combined filtrate and washings were carefully treated with magnesium oxide paste until distinctly alkaline to phenolphthalein and distilled for the nitrogen existing as ammonia. The distillation was continued until the volume in the nitrogen flask was about 50 cc. After cooling, the contents of the flask were filtered and thoroughly washed until the filtrate was neutral to phenolphthalein. The magnesium oxide precipitate was returned to the nitrogen flask and the amount of nitrogen determined by the Kjeldahl method.

TABLE V.—NITROGEN GIVEN OFF AS AMMONIA DURING THE CONCENTRATION OF THE ALKALINE FILTRATE.

	Nitrogen as ammonia.		
Laboratory No.	First distillation. Per cent.	Second distillation. Per cent.	Total. Per cent.
101*a*	0.96	0.04	1.00
101*b*	0.96	0.03	0.99
101*c*	0.97	0.05	1.02
101*d*	1.00	none	1.00
101*e*	0.99	0.03	1.02
307*a*	1.00	0.08	1.08
307*b*	1.08	0.01	1.09
302*a*	0.76	0.02	0.78
302*b*	0.74	0.05	0.79
110*a*	1.14	0.06	1.20
110*b*	1.15	0.07	1.22
110*c*	1.19	0.04	1.23

The filtrate and washings from the magnesium oxide precipitate were distilled to about 100 cc. and the ammonia in the distillate determined and added to the amount found in the first distillation, the total nitrogen thus obtained being reported as "Nitrogen as Ammonia." That this modification was necessary is shown by the data given in Table V.

The concentrated filtrate from the magnesium oxide precipitate was diluted to a volume of 200 cc., and 100 cc. of this solution, representing 0.5 gram of the proteid were acidified with sulphuric acid and then precipitated with 30 cc. of phosphotungstic acid as directed by Osborne and Harris. The combined filtrate and washings from the phosphotungstic acid precipitate were diluted to 500 cc. and the nitrogen determined in 100 cc. portions. As a check on these determinations the total nitrogen was determined in 50 cc. portions of the filtrate from the magnesium oxide precipitate. The character of the results thus obtained may be judged from the data given in the following table:

TABLE VI.—RESULTS ILLUSTRATING THE METHOD USED IN CHECKING THE DETERMINATION OF THE BASIC AND NON-BASIC NITROGEN.

Laboratory No.	Nitrogen in phosphotungstic acid precipitate. Per cent.	Nitrogen in filtrate from phosphotungstic acid precipitate. Per ct.	Sum of the nitrogen in the two previous columns. Per cent.	Total nitrogen in the filtrate from MgO precipitate. Per cent.	Difference. Per cent.
101*a*	4.47	10.18	14.65	14.65	none
101*b*	4.52	9.87	14.39	14.48	0.09
115*a*	4.54	10.02	14.56	14.50	0.06
115*b*	4.34	9.81	14.15	14.34	0.19
110*a*	4.49	8.50	12.99	13.53	0.54
110*b*	4.48	8.70	13.18	13.51	0.33

Composition and Properties of Fractional Coagula of Water Extracts of Flesh.—For this purpose a water extract was prepared as previously described from 1587 grams of selected, lean, beef round taken from a prime export steer. The water-free substance of this sample of meat equaled 26.30 per cent. of the fresh substance.

Eleven liters of the clear filtered extract were obtained which had a total acidity equivalent to 0.75 per cent. calculated as lactic acid. The acidity of this entire extract was reduced to 0.34 per cent. upon the same basis by the addition of the required

quantity of a standard solution of potassium hydroxide. The partial neutralization of the extract produced a considerable quantity of a precipitate which was nearly white in color. It was removed by filtration and thoroughly washed first with water, then with strong alcohol and finally with ether. The residue thus obtained was rubbed through a sieve while drying it from the ether and it was then given laboratory No. 302*a*. It was dried to constant weight at a temperature of 104°. The residue thus dried weighed 3.8071 grams and contained 16.44 per cent. of nitrogen.

The filtrate from the above precipitate which was produced by neutralization was heated for two hours at a constant temperature of 50°. A considerable amount of a nearly white coagulum was formed which was removed by filtration and thoroughly washed with water, alcohol and ether. The coagulum thus obtained, which was given laboratory No. 302*b*, weighed 7.6705 grams after drying to constant weight at 104° and contained 16.31 per cent. of nitrogen.

The filtrate from the above coagulum No. 302*b* was again heated for two hours at 50°. An additional coagulum which was almost white was obtained and after removal by filtration was thoroughly washed as above. It was given laboratory No. 302*c*. The proteid thus obtained weighed 3.6860 grams after it had been dried to constant weight at 104° and in this condition it contained 16.05 per cent. of nitrogen.

The solution from the second coagulum at 50° was again heated for two hours at this temperature. The nearly white coagulum which resulted was separated by filtration and thoroughly washed with water, alcohol and ether. The quantity of proteid thus obtained equaled 3.9308 grams after drying to constant weight at 104°. It contained 16.46 per cent. of nitrogen, and was labeled 302*d*.

The three fractions Nos. 302*b*, 302*c* and 302*d* of the coagulum occurring at 50° were made into a composite sample for the purpose of studying the hydrolytic products produced by boiling with hydrochloric acid

Hausmann's method as modified by Osborne and Harris gave the following results for the proportion of nitrogen in the different forms of combination occurring in the decomposition products.

TABLE VII.—PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN THE PROTEID OF A WATER EXTRACT OF FLESH COAGULATING AT 50°.

	Per cent.
Nitrogen as ammonia	1.05
Basic nitrogen	4.44
Non-basic nitrogen	10.48
Nitrogen in magnesium oxide precipitate	0.33[1]
Nitrogen in the humin substances	.....
Total nitrogen by summation	16.30
Total nitrogen in proteid	16.27

By the coagulation of the three fractions Nos. 302*b*, *c*, *d*, of the proteid at a temperature of 50° the acidity of the solution had increased 80 per cent. The acidity of the entire extract was again reduced to 0.34 per cent. calculated as lactic acid, by the addition of a standard solution of potassium hydroxide. The neutralization produced a light gray precipitate which was separated from the solution by filtration, and then thoroughly washed with water, alcohol and ether. The precipitate which was labeled 302*e*, weighed 1.3058 grams after drying at 104° and contained 16.09 per cent. of nitrogen.

The filtrate from the above precipitation which was produced by neutralization was heated for four hours at a constant temperature of 50°. A further coagulum of a pinkish white color was obtained which was labeled 302*f*. It weighed 1.400 grams after it had been dried to constant weight at 104° and an analysis showed that it contained 15.62 per cent. of nitrogen. Mention should be made of the low content of nitrogen in this sample notwithstanding the fact that the nitrogen in the precipitate obtained by partial neutralization is about as high as it is in the preceding coagula. This agrees with results obtained in several other cases; namely, that heating following neutralization or partial neutralization gives a coagulum of less nitrogen content than the precipitate produced by the addition of the alkali. Further study of this problem is now being made.

The filtrate from the last coagulum was heated for four hours at a temperature of 75°, and as a result a large quantity of a grayish white coagulum was produced. After filtering, washing and drying as usual this fraction weighed 17.2103 grams and contained 16.54 per cent. nitrogen. It was given laboratory

[1] Includes the nitrogen contained in the humin substances.

No. 302*g*. This coagulum separating at 75° gave the following results by hydrolytic decomposition with hydrochloric acid.

TABLE VIII.—PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN THE PROTEID OF A WATER EXTRACT OF FLESH COAGULATING AT 75°.

	Per cent.
Nitrogen as ammonia	1.00
Basic nitrogen	4.91
Non-basic nitrogen	9.80
Nitrogen in magnesium oxide precipitate	0.22
Nitrogen in the humin substances	0.05
Total nitrogen by summation	15.98
Total nitrogen in proteid	15.54

By heating the filtrate from 302*g* for four hours at 75°, a small quantity of a finely divided dark brown coagulum resulted. After separation and purification this coagulum weighed 0.9451 grams and by analysis it was found to contain 16.22 per cent. of nitrogen. This fractional coagulum was given laboratory No. 302*h*.

The filtrate from No. 302*h* was heated for eight hours at a temperature varying from 85° to 90°. A large quantity of a finely divided brick-red coagulum was formed which was removed by filtration and washed thoroughly with water, alcohol and ether. After drying at 104° this coagulum weighed 5.330 grams and contained 16.44 per cent. of nitrogen. It was labeled 302*i*.

This coagulum separating at 85° gave the following results by hydrolytic decomposition with hydrochloric acid.

TABLE IX.—PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN THE PROTEID OF A WATER EXTRACT OF FLESH COAGULATING AT 85°.

	Per cent.
Nitrogen as ammonia	0.79
Basic nitrogen	4.11
Non-basic nitrogen	10.94
Nitrogen in magnesium oxide precipitate	0.53
Nitrogen in the humin substances	0.12
Total nitrogen by summation	16.49
Total nitrogen in proteid	16.44

The filtrate from the last precipitate was now exactly and completely neutralized with a standard solution of potassium hydroxide. No precipitation was noticeable at first but upon

TABLE X.—SUMMARY OF THE ABOVE STUDY OF FRACTIONAL COAGULATION.

Laboratory No.	Condition of the formation of the precipitates in the filtrate from the preceding precipitate.	Appearance of precipitates.	Weight of precipitates. Grams.	Nitrogen contained in the precipitates. Per cent.	Nitrogen factor.	Per cent. of each precipitate of the total weight of precipitates.	Per cent. of each precipitate calculated to the air-dried sample of meat.	Results of hydrolysis. Nitrogen as ammonia. Per cent.	Basic nitrogen. Per cent.	Non-basic nitrogen. Per cent.	Nitrogen in magnesium oxide precipitate. Per cent.	Nitrogen in the humin substances. Per cent.	Total nitrogen by summation. Per cent.	Nitrogen in the proteid. Per cent.
302*a*	By partial neutralization.....	Nearly white.	3.8071	16.44	6.11	8.37	0.91	...	...	....	...	...	....	....
302*b*	By coagulation, at 50°, for 2 hours	Nearly white.	7.6705	16.31	6.10	16.86	1.83							
302*c*	By coagulation, at 50°, for 2 hours	Nearly white.	3.6860	16.05	6.23	8.10	0.88	1.05	4.44	10.48	0.33[1]	...	16.30	16.27
302*d*	By coagulation, at 50°, for 2 hours	Nearly white.	3.9308	16.46	6.08	8.64	0.94							
302*e*	By partial neutralization again..	Light gray.	1.3058	16.09	6.22	2.87	0.31	...	...	....	...	...	....	....
302*f*	By coagulation, at 50°, for 4 hours	Pinkish white.	1.4000	15.62	6.40	3.08	0.33	...	...	....	...	...	....	....
302*g*	By coagulation, at 75°, for 4 hours	Gray-white.	17.2103	16.54	6.04	37.82	4.12	1.00	4.91	9.80	0.22	0.05	15.98	16.54
302*h*	By coagulation, at 75°, for 4 hours	Dark brown.	0.9451	16.22	6.09	2.08	0.23	...	...	....	...	...	....	....
302*i*	By coagulation, at 85°–90°, for 8 hours.....	Brick-red.	5.3320	16.44	6.08	11.72	1.27	0.79	4.11	10.94	0.53	0.12	16.49	16.44
302*j*	By complete neutralization and heat.....	Gray-white.	0.2160	2.91	...	0.47	0.05	...	...	....	...	...	....	....
			45.5036	16.24	6.10[2]	100.00	10.97	...	...	....	...	...	....	....

[1] Contains also the nitrogen of the humin substances.

[2] Taking into consideration the relative amounts of precipitates.

heating on the water-bath a small quantity of a light colored flocculent precipitate resulted which was removed by filtration and thoroughly washed in the usual manner. After drying this precipitate to constant weight at 104° it weighed 0.2160 gram and contained 2.91 per cent. of nitrogen. It was given laboratory No. 302*j*.

The conclusions to be drawn from the results of the experiments described in this connection are as follows:

First, by a study of the data presented in the above tables it will be found that the total proteid matter separated by neutralization and coagulation amounted to 2.86 per cent. of the weight of the flesh taken for the experiment. The proteids precipitated by neutralization formed 0.34 per cent., the proteids separated by coagulation at a temperature of 50° formed 1.05 per cent., while the proteids coagulating at 75°, 1.14 per cent. and the proteids coagulating at 85° formed 0.34 per cent. of the weight of the flesh taken for the study.

Expressed in another way, of the total proteid material of a cold water extract of raw flesh that is precipitated by neutralization and coagulation, 11.71 per cent. is separated by neutralization, 36.65 per cent. is precipitated by coagulation at a temperature of 50°, 39.93 per cent. coagulates at a temperature of 75° and 11.71 per cent. is precipitated by coagulation at a temperature of 85°.

Second, it is apparent that the chemical composition of the different coagula, at least so far as nitrogen content is concerned, is remarkably constant. The nitrogen in the several fractions varies from 15.62 to 16.54 per cent. not including the fraction 302*j* which was proved to be composed chiefly of calcium phosphate. The carbon, hydrogen, sulphur, phosphorus and ash contained in these coagula will be determined.

Third, it is evident that the results of the hydrolysis of the several fractions of the proteids coagulating at 50°, 75° and 85° indicate clearly that they are quite similar as regards their chemical constitution. Further study of the hydrolytic products of these different coagula is under way.

Fourth, the coagula obtained at temperatures of 48° to 50° from aqueous extracts of flesh are soluble in very dilute solutions of potassium and sodium hydroxides. They are reprecipitated

upon slightly acidifying with very dilute hydrochloric acid but soluble in the latter acid upon the addition of a very slight excess. This latter solution in hydrochloric acid is precipitated by the addition of sodium chloride to somewhat less than one-fourth saturation. The precipitate so formed is soluble in water and can again be reprecipitated from solution by the addition of sodium chloride.

The coagula obtained at 75° and at 85° from aqueous extracts of flesh are both soluble in a 0.5 per cent. solution of potassium hydroxide, forming a greenish brown solution. They are not as readily soluble in alkaline solutions as are the coagula formed at 50°.

A STUDY OF THE INFLUENCE OF ACIDITY UPON FRACTIONAL COAGULATION OF WATER EXTRACTS OF FLESH.

Previous work[1] done in this laboratory, together with the preliminary experiments which were made in connection with the research here reported, indicated very clearly that there is a close relationship existing between the act of the coagulation of the proteids and the acidity of the water extracts of flesh. In order to determine exactly these conditions as regards the degree of acidity best suited to effect the fractional coagulation of the proteids of aqueous extracts of flesh the following experiment was undertaken.

A water extract was prepared by the usual method from 1701 grams of lean beef round taken from a three-year-old heifer. Of the 10 liters of the extract which were prepared, 4 liters of the first and most concentrated portion were taken for the experiment here described. One hundred cubic centimeters of this extract contained 1.8964 grams of total solids dried at 104° and 0.2498 gram of nitrogen. The nitrogen in the extract therefore formed 13.18 per cent. of the total dry substance of the same. The total acidity of the entire extract calculated as lactic acid was equivalent to 1.18 per cent. of the fresh substance. Phenolphthalein was used as the indicator.

From this water extract five series of samples each measuring 200 cc. were taken. The samples were transferred to 500 cc. Kjeldahl flasks and treated as follows:

[1] This Journal, **27**, 668 (1905).

(A) Triplicate samples of 200 cc. each. The acidity of the original extract was not changed. Acidity equaled 1.18 per cent.

(B) Triplicate samples of 200 cc. each. The acidity of the extract was reduced one-fourth. Acidity equaled 0.88 per cent.

(C) Triplicate samples of 200 cc. each. The acidity of the original extract was reduced one-half. Acidity equaled 0.59 per cent.

(D) Triplicate samples of 200 cc. each. The acidity of the original extract was reduced three-fourths. Acidity equaled 0.30 per cent.

(E) Triplicate samples of 200 cc. each. The acidity of the original extract was completely neutralized. The reaction of these solutions was neutral to phenolphthalein.

Upon adding the necessary amounts of a standard solution of potassium hydroxide to effect the neutralization above mentioned the following results were produced. In the B's there were only faint precipitates which were easily and rapidly filtered from the solutions.

In the C's there were small precipitates which collected into masses resembling coagula. These precipitates were easily and rapidly removed by filtration.

In the D's there were large quantities of precipitates which were separated fairly well by filtration but not so readily as those from B and C.

In the E's the precipitates produced by neutralization were large but they were separated only tardily by filtration.

The precipitates produced above were washed with water and the filtrates and washings collected in Kjeldahl flasks. The washed precipitates were returned to the flasks in which they had been precipitated and the nitrogen which they contained determined by the Kjeldahl method.

The filtrates from the above precipitates, together with solutions A to which no potassium hydroxide solution had been added, were all subjected to the following series of treatments. After each treatment the precipitates were separated by filtration and washed and then the nitrogen determined in them. The averages of the analytical results are given in Table XI.

First, they were heated for two hours at 50°. The triplicate samples of A gave turbid solutions which required twelve hours to filter and wash. The solutions B, C, and D all filtered rapidly, giving clear filtrates. Solution E filtered slowly but gave a clear filtrate.

Second, they were again heated for two hours at 50°.

Third, the solutions were again heated for two hours at 50°.

Fourth, they were heated at 50° for four hours.

Fifth, the solutions were heated at 50° for five and one-half hours.

Sixth, the solutions were then heated at 60° for two hours.

Seventh, they were heated at 60° for four hours.

Eighth, the solutions were further heated at 60° for four and one-half hours.

Ninth, they were heated at 70° for three hours.

Tenth, the solutions were further heated at 70° for three hours but as no precipitation occurred the temperature was increased to 80°. It required one hour for the temperature of the solutions to reach 80°. They were then kept at 80° for three hours.

Eleventh, the filtrates from the above were placed in a bath of boiling water. They became turbid in a few minutes. The solutions were maintained at this temperature (96°) for three hours.

Twelfth, the filtrates were all concentrated on the water-bath to a volume of about 50 cc. and the resulting precipitates removed by filtration.

Thirteenth, the filtrates and washings from the above were diluted to definite volume and an aliquot part taken for the determination of albumoses.

Fourteenth, a second aliquot part of the above solution was used for the determination of the peptones.

Fifteenth, a third aliquot portion of the solution was used for the determination of the total nitrogen so that from the data obtained under the last two headings the quantity of the nitrogenous extractives could be estimated.

A condensed summary of the analytical results of this experiment is given in the following table:

TABLE XI.—SUMMARY SHOWING THE INFLUENCE OF THE DEGREE OF ACIDITY UPON THE FRACTIONAL COAGULATION OF A COLD WATER EXTRACT OF FLESH.

(Results expressed in grams of nitrogen contained in total extract, 4000 cc.)

Nitrogen obtained.	A. Without neutralization. Grams.	B. One-fourth neutralization. Grams.	C. One-half neutralization. Grams.	D. Three-fourths neutralization. Grams.	E. Complete neutralization. Grams.	Time required for coagulation. Hours.
By addition of alkali	0.547[1]	0.099	0.240	0.469	0.514	..
By coagulation at 50°	2.768	2.497	2.277	2.478	2.718	15½
By coagulation at 60°	0.557	1.734	1.902	1.528	1.293	10½
By coagulation at 70°	0.293	0.553	0.404	0.258	0.250	7
By coagulation at 80°	0.992	0.721	0.873	0.794	0.660	3
By coagulation at 96°	0.066	0.233	0.162	0.106	0.108	39
Total by coagulation	4.677	5.738	5.618	5.165	5.030	..
By concentration	0.024	0.039	0.066	0.111	0.177	..
Albumoses and peptones	0.251	0.328	0.724	0.697	0.850	..
Extractives	3.530	3.778	3.547	3.504	3.383	..
Total nitrogen by summation	9.029	9.983	10.196	9.946	9.954	..

This experiment which was undertaken to determine the influence of acidity upon the fractional coagulation of water extracts of flesh leads to the following conclusions:

First, that it is necessary to effect at least partial neutralization of aqueous extracts of flesh in order that the different coagula may be of such a nature that they may be readily and completely removed by filtration from the liquids in which they were coagulated. In all cases, the extracts which had not been partially neutralized gave turbid solutions which required twelve to twenty-four hours for filtration and washing. In fact it was found to be impossible to complete successfully the above series of tests upon the solutions that had not been neutralized. That being the case after they had been heated to a temperature of 80° and the several fractions below this temperature had been with much difficulty incompletely removed it was found necessary to partially neutralize them before proceeding further with the experiment. As a result of this treatment the solutions which from the first had continually filtered very slowly, giving turbid filtrates, now filtered rapidly and gave perfectly clear filtrates.

Second, that one-fourth neutralization to phenolphthalein of the natural acidity of cold water extracts of flesh gave such con-

[1] In A, the neutralization was effected after heating to 80°.

ditions in the resulting solutions, that the separation of the coagulable proteids is more complete than it is if further neutralization of the acidity is produced. The total nitrogen in the combined fractions of the coagulated proteid in the 4 liters of extract in question for the different degrees of neutralization was as follows: One-fourth neutralization 5.738 grams, one-half neutralization 5.618 grams, three-fourths neutralization 5.165 grams and complete neutralization 5.030 grams. It is thus apparent that if the acidity of these solutions is decreased beyond that of one-fourth neutralization, there is a proportional decrease in the amount of proteids separated by coagulation.

Third, the data here presented point clearly to the conclusion that the more nearly the natural acidity of water extracts of flesh is neutralized before separating the coagulable proteids by the method here indicated, the greater is the quantity of proteids obtained as albumoses and peptones.

C. THE PROTEIDS OF FLESH INSOLUBLE IN COLD WATER BUT SOLUBLE IN A 10 PER CENT. SOLUTION OF AMMONIUM SULPHATE.

After the complete removal of the water-soluble constituents of flesh, a considerable portion of the residue was found to be soluble in a 10 per cent. solution of ammonium sulphate. Quantitative experiments have shown that a 10 per cent. solution of ammonium sulphate removes from the residue remaining after the complete extraction of flesh with water, 3.00 per cent. of proteid calculated upon the basis of the fresh substance of the flesh. The extraction of the residue insoluble in water, with the ammonium sulphate solution, was conducted in the same manner as the extraction of the original flesh with cold water. The extraction was continued until the last filtrate failed to give the biuret reaction for proteid. The ammonium sulphate extract after it was carefully strained through cheese-cloth without pressure, consisted of a turbid solution that could not be filtered through paper. By allowing this turbid solution to stand over night in cold storage, a gray-white flocculent precipitate separated, leaving a perfectly clear solution. The precipitate was washed several times by decantation with a 10 per cent. solution of ammonium sulphate. After this treatment the precipitate was collected upon a filter and thoroughly washed with water until entirely free from sulphates and then digested in alcohol and finally washed with alcohol and ether. The preparation thus

obtained was dried to constant weight at a temperature of 104°. Two samples of this proteid were prepared from different samples of flesh. Upon analysis the first sample (No. 110) gave 14.52 per cent. of nitrogen and 1.60 per cent. of sulphur. The second sample (No. 308*a*) contained 15.15 per cent. of nitrogen and 1.60 per cent. of sulphur. Hausmann's method as modified by Osborne and Harris gave the following results for the proportion of nitrogen in the different forms of combination occurring in the hydrolytic products.

TABLE XII.—PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS, IN THE PROTEIDS SEPARATED UPON STANDING FROM A 10 PER CENT. AMMONIUM SULPHATE EXTRACT OF FLESH.

	First preparation. No. 110.	Second preparation. No. 308*b*.
Nitrogen as ammonia	1.16	1.19
Basic nitrogen	4.43	4.01
Non-basic nitrogen	8.65	9.33
Nitrogen in magnesium oxide precipitate	0.15	0.18
Nitrogen in the humin substances	0.10	0.14
Total nitrogen by summation	14.49	14.85
Total nitrogen in proteid	14.52	15.15

The clearly filtered ammonium sulphate extracts from which the above preparations separated upon standing are capable of undergoing fractional coagulation. A portion of one of the above ammonium sulphate extracts was maintained at a temperature of 50° for ten days. The coagulum formed was separated each day. A small quantity of coagulum would form within a few hours after each filtration. The coagula forming at 50° were combined and given laboratory No. 130. The filtrate obtained from the coagula separating at 50°, was kept at a temperature of 60° for a considerable time. A small coagulum formed which as yet has not been examined further. The filtrate from this coagulum at 60°, when heated to the boiling-point of water, gave relatively a considerable quantity of coagulated proteid which was separated, thoroughly washed with water, alcohol and ether and given laboratory No. 131.

Portions from each of the 10 per cent. ammonium sulphate extracts were completely coagulated at the temperature of boiling water, no fractions being separated at lower temperatures. The coagula were thoroughly washed with water and dilute alcohol until all sulphates were removed. They were then dried with

alcohol and ether and given laboratory Nos. 109 and 308*a*. These samples together with Nos. 130 and 131 mentioned above were dried to constant weight at a temperature of 104°, and the nitrogen was determined in each with the following results: No. 109, 15.44 per cent.; No. 308*a*, 15.83 per cent.; No. 130, 15.94 per cent.; and No. 131, 15.11 per cent. The sulphur in No. 109 equaled 2.25 per cent. and that in No. 308*a* amounted to 1.82 per cent.

The proportion of nitrogen in the different forms of combination that occur in the decomposition products produced by boiling preparations Nos. 109 and 308*a* with hydrochloric acid was determined.

TABLE XIII.—PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN THE PROTEIDS SEPARATED UPON COMPLETE COAGULATION FROM A 10 PER CENT. AMMONIUM SULPHATE EXTRACT OF FLESH.

	First preparation. No. 109.	Second preparation. No. 308*a*.
Nitrogen as ammonia	1.01	0.92
Basic nitrogen	5.40	4.81
Non-basic nitrogen	8.88	9.66
Nitrogen in magnesium oxide precipitate	0.14	0.22
Nitrogen in the humin substances	0.01	0.04
Total nitrogen by summation	15.44	15.65
Total nitrogen in proteid	15.45	15.83

For the purpose of comparison a meat residue (laboratory No. 301) left after complete extraction with cold water and a residue left after complete extraction first with water and then with 10 per cent. ammonium sulphate solution (laboratory No. 307*a*) were hydrolyzed. The results are given in the following table.

TABLE XIV.—PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS IN THE EXTRACTED RESIDUES OF FLESH.

	Residue. No. 301.	Residue. No. 307*a*.
Nitrogen as ammonia	1.25	1.08
Basic nitrogen	4.75	4.52
Non-basic nitrogen	10.01	10.05
Nitrogen in magnesium oxide precipitate	0.21	0.18
Nitrogen in the humin substances	0.07	0.05
Total nitrogen by summation	16.29	15.88
Total nitrogen in proteid	16.32	16.25

The flesh residues after the removal of the water extracts and the 10 per cent. ammonium sulphate extracts are readily freed

from the ammonium sulphate by washing with cold water. The washings so obtained contain only a mere trace of proteid matter. The washed residues after dehydrating with alcohol and ether are nearly white, having, however, as a rule a very slight grayish or yellowish tint. These residues closely resemble asbestos in general appearance. Two such residues have been analyzed for nitrogen and sulphur, and in one case the analogous analytical data were obtained for the residue after complete extraction with cold water but immediately before the extraction with the 10 per cent. solution of ammonium sulphate. The analytical data for the residue No. 203 which had been completely freed from the constituents soluble in cold water but which had not been extracted with the ammonium sulphate solution were as follows: Nitrogen 15.90 per cent., sulphur 1.05 per cent. and ash 0.70 per cent. The analogous data for the same original sample after complete extraction with a 10 per cent. ammonium sulphate solution, laboratory No. 214, were as follows: Nitrogen 15.88 per cent., sulphur 1.09 per cent. and ash 0.29 per cent.

A second residue representing the portion of flesh insoluble both in cold water and a 10 per cent. solution of ammonium sulphate (laboratory No. 307*a*) gave upon analysis 16.25 per cent. of nitrogen, 1.15 per cent. of sulphur and ash 0.39 per cent. The results of the hydrolysis of this same residue are given above, page 497.

This study of the proteids of flesh insoluble in cold water but soluble in a 10 per cent. solution of ammonium sulphate leads to the following conclusions:

First, that after flesh has been completely freed from proteids soluble in cold water, it contains two classes of proteid substances, namely, those which are soluble in a 10 per cent. solution of ammonium sulphate and those which are insoluble in this medium.

Second, that there could be separated from the turbid ammonium sulphate extract of flesh at least two individual proteids or groups of proteids which differ in physical properties and to some extent in chemical composition. These two substances or groups of substances differed from each other physically in that one of them, even if it was at first completely soluble in the 10 per cent. ammonium sulphate solution, which is doubtful, did not remain long in solution but separated from the same upon standing, while the other substance was readily and completely soluble in

this medium. These two proteid products separated from the turbid ammonium sulphate extract differ from each other in chemical composition. The one separating upon standing, containing a smaller percentage of total nitrogen (average 14.84), and sulphur (average 1.60) and also yielding upon hydrolysis less basic nitrogen (average 4.22) than the one which was separated by coagulation from the 10 per cent. ammonium sulphate extract. The analogous analytical data obtained for the latter substance were as follows: Total nitrogen 15.55 per cent., sulphur 2.04 per cent. and basic nitrogen 5.11 per cent. Of course these data regarding the composition of these two products must be considered more or less of a tentative nature until they are confirmed by further analyses.

Third, the percentage of sulphur in the two different proteid bodies obtained from the ammonium sulphate extract is very decidedly higher than it is in any of the other proteids which we have as yet separated from flesh. The highest sulphur content of any other proteid preparations of flesh so far obtained is 1.15 per cent. In the light of Osborne's work upon the vegetable proteids, it is probable that the higher content of sulphur in these products which are obtained from the ammonium sulphate solution is due to the fact that the solvent has reacted with the basic proteid molecule with the formation of a salt-like product. Further study is now being made in this connection to determine whether or not this is the case.

D. THE PROTEIDS OF FLESH INSOLUBLE IN COLD WATER, AND IN A 10 PER CENT. SOLUTION OF AMMONIUM SULPHATE, BUT SOLUBLE IN A COLD SOLUTION OF N/20 POTASSIUM HYDROXIDE.

The moist residues of flesh which remain after the complete extraction of fresh beef flesh, first, with cold water and second with a 10 per cent. solution of ammonium sulphate, when digested with a solution of N/20 potassium hydroxide, swell up at first to a semi-solid, jelly-like mass. Upon standing for several hours, most of the proteid apparently is dissolved but the resulting solution always remains turbid. It is practically impossible to filter the solutions through filter-paper. By straining them through cheese-cloth and through muslin the solid particles are removed and the extent of the turbidity is decreased, but a clear transparent solution is not obtained.

The N/20 potassium hydroxide solution of the flesh residue thus obtained may be treated with dilute hydrochloric acid until it is just neutral to phenolphthalein without causing a precipitation of the proteid. Again, the alkaline solution may be rapidly poured into an acid solution of such strength that the resultant solution is N/20 hydrochloric acid, without causing the precipitation of the proteid materials. When the alkaline solution is made distinctly acid to phenolphthalein the proteids separate as a white flocculent precipitate. If the proper quantity of hydrochloric acid is added, the liquid above the separated proteids becomes perfectly clear, the precipitate settles rapidly and it can be washed readily by decantation.

One residue of flesh which was left after extracting completely 10 pounds of lean beef round, successively with cold water, with a 10 per cent. solution of ammonium sulphate and with cold water to completely remove the last-mentioned solvent, was treated with a N/20 solution of potassium hydroxide. The resulting solution was filtered through muslin and precipitated by the addition of the proper amount of hydrochloric acid. The precipitate thus produced was washed thoroughly by decantation and dissolved in an N/80 solution of potassium hydroxide. This second solution of the proteid in the alkali was filtered through cheese-cloth and then reprecipitated with hydrochloric acid. The proteid thus precipitated was washed thoroughly by decantation and then upon a Büchner funnel by the use of a filter pump until the wash-water from the same gave no test for chlorides.

The large quantity of proteid obtained by the above treatment was divided approximately into six equal portions and each portion was further purified by the treatment indicated below.

Portion I was repeatedly extracted first with strong alcohol and then with ether. The proteid was air-dried and given laboratory No. 111.

Portion II was dissolved in a solution of N/80 potassium hydroxide and the solution resulting was filtered through cloth. It was then precipitated by the addition of the proper quantity of hydrochloric acid. The precipitated proteid was washed with water until entirely free from chlorides and then with alcohol and ether to remove fat and to properly air-dry the sample. The fraction of the proteid thus purified was labeled laboratory No. 112.

Portion III was twice subjected to the above treatment, that is, it was dissolved in the dilute alkali two times and precipitated by hydrochloric acid each time. The resulting product was thoroughly washed with water, alcohol and ether and given laboratory No. 113.

Portion IV was dissolved in an N/80 solution of potassium hydroxide and the solution produced filtered through cloth. The filtered solution was poured into a solution of hydrochloric acid of such a strength that the solution resulting would contain sufficient acid after the neutralization of the potassium hydroxide to form a N/40 solution of hydrochloric acid. The solution thus formed was not entirely clear but no precipitate separated from the same upon standing over night. The proteid contained in this acid solution was precipitated by the addition of a proper amount of potassium hydroxide. The precipitate was thoroughly washed with water, alcohol and ether and labeled No. 114.

Portion V was dissolved in potassium hydroxide and the solution thus formed poured into hydrochloric acid as described above for portion IV. The proteid contained in the acid solution in this case was, however, precipitated by the addition of one-fourth of its volume of a saturated solution of sodium chloride, containing enough of hydrochloric acid to make the acidity of the same equal to N/40. The resulting proteid precipitate was thoroughly washed with water by decantation and then dissolved in a solution of N/80 potassium hydroxide. This solution was strained through cheese-cloth and then precipitated by the addition of hydrochloric acid. The precipitate thus purified was washed very thoroughly with water, alcohol and ether. This preparation was labeled No. 115.

Portion VI was treated in the first place as portion V with the exception of the last operation. Instead of precipitating the alkaline solution directly with hydrochloric acid, it was poured into a solution of hydrochloric acid of such a strength that the solution resulting contained sufficient acid after the neutralization of the potassium hydroxide to form a N/40 solution of hydrochloric acid. The proteid dissolved in the excess of acid was precipitated by the addition of a solution of potassium hydroxide. The resulting precipitate was completely washed with water, alcohol and ether and then given laboratory No. 116.

TABLE XV.—CHEMICAL COMPOSITION OF DIFFERENT PREPARATIONS OF PROTEIDS OF FLESH INSOLUBLE IN WATER AND A 10 PER CENT. SOLUTION OF AMMONIUM SULPHATE BUT SOLUBLE IN A N/20 SOLUTION OF POTASSIUM HYDROXIDE.

Lab. No.	Method of purification.	Carbon. Per cent.	Hydrogen. Per cent.	Nitrogen. Per cent.	Sulphur. Per cent.	Ash. Per cent.
111	Dissolved two times in KOH, precipitated by HCl	51.90	7.37	15.82	1.14	0.52
112	Dissolved three times in KOH, precipitated by HCl	51.56	7.40	15.51	1.10	0.52
113	Dissolved four times in KOH, precipitated by HCl	51.56	7.43	15.26	1.11	0.55
114	Dissolved two times in KOH, poured into excess HCl, precipitated by KOH	51.26	7.38	15.45	1.09	0.50
115	Dissolved two times in KOH, poured into excess HCl, precipitated by NaCl, dissolved in KOH, precipitated by HCl	51.39	7.42	15.55	1.12	0.40
116	Dissolved two times in KOH, poured into excess HCl, precipitated by NaCl, dissolved in KOH, poured into excess HCl, precipitated by KOH	51.71	7.34	15.38	1.05	0.55
	Average	51.56	7.39	15.50	1.10	0.51

About 45 grams of air-dried proteid were obtained from each of the above treatments. Portions of each preparation were dried to constant weight at a temperature of 104° and then completely analyzed with the results obtained in the above table.

The analytical results of this experiment indicate clearly that the proteid material of flesh insoluble in water and in a 10 per cent. solution of ammonium sulphate, but soluble in a N/20 solution of potassium hydroxide solution, has the same chemical composition even when separated from the solvent by different means and purified by widely differing treatment.

While the results undoubtedly prove that we are dealing here with a comparatively pure proteid material, they do not by any means necessarily prove that the same is a single proteid substance, since it is often true that proteids differing greatly in physical properties and chemical behavior have practically the same chemical composition.

A composite sample was made of the preceding samples Nos. 111 to 116 inclusive and the resulting sample was carefully dried at 104°, and then hydrolyzed as usual with a 20 per cent. solution of hydrochloric acid. The results are given in the following table:

TABLE XVI.—PERCENTAGE OF NITROGEN IN THE DIFFERENT GROUPS, IN THE COMPOSITE SAMPLE (NOS. 111 TO 116, INCLUSIVE) OF THE PROTEIDS OF FLESH NOT SOLUBLE IN WATER OR AMMONIUM SULPHATE BUT SOLUBLE IN DILUTE POTASSIUM HYDROXIDE.

	Per cent.
Nitrogen as ammonia	0.98
Basic nitrogen	4.49
Non-basic nitrogen	9.92
Nitrogen in magnesium oxide precipitate	0.14
Nitrogen in the humin substances	0.07
Total nitrogen by summation	15.60
Total nitrogen in the proteid	15.50

A more detailed study of the decomposition products resulting upon the hydrolysis of the different proteid preparations obtained from the potassium hydroxide solution of the residue of flesh insoluble in water and in a 10 per cent. solution of ammonium sulphate is being made in this laboratory.

CONCLUSIONS.

(1) The total acidity of aqueous extracts of flesh varies between comparatively wide limits, the minimum being 0.66 per cent.,

the maximum 1.07 per cent. and the average 0.85 per cent., calculated as lactic acid and expressed in terms of the fresh substance of the meat.

(2) The residues of flesh which are left after the complete extraction with cold water are distinctly acid to litmus and to phenolphthalein.

(3) On the other hand, the insoluble proteids of flesh upon digestion with a N/10 solution of hydrochloric acid at the ordinary temperature combine with the latter, neutralizing its acid properties.

(4) Analyses made in this laboratory prove that 13.56 per cent. of the total proteid existing in lean beef flesh is soluble in cold water. Of this total soluble proteid 90.04 per cent. is in a form which is coagulable by heat from a neutral solution, 8.40 per cent. exists as albumoses and a very small quantity apparently exists in the form of peptones. It is not at present supposed that the albumoses and peptones exist as such in the original flesh.

(5) During the coagulation of an aqueous extract of flesh there is an increase in the acidity of the same. Reduction of the acidity of aqueous extracts of flesh facilitates coagulation of the proteids. One-fourth neutralization (to phenolphthalein) of the natural acidity of the cold water extracts gives such conditions in the resulting solutions, that the separation of the coagulable proteids is more complete than it is if further neutralization of the acidity is produced. The partial neutralization of the acidity of such extracts at the ordinary temperature causes the precipitation of some proteid matter.

(6) There are no well defined degrees of temperature at which different coagula of aqueous extracts of flesh separate. The complete removal of the proteid coagulating at any fixed definite temperature requires long application of heat. Further, the lower the temperature at which a coagulum is separated the longer the time of heating required to effect complete coagulation.

(7) Of the total proteid of a cold water extract of flesh, that is, precipitated by neutralization and coagulation, 11.71 per cent. is separated by neutralization, 36.65 per cent. is precipitated by coagulation at a temperature below 50°, 39.93 per cent. coagulates between 51° to 75° and 11.71 per cent. is precipitated by coagulation at a temperature between 76° to 85°.

(8) The chemical composition of the different fractional coagula of the aqueous extracts of raw flesh is remarkably constant. They are also quite similar as regards their chemical constitution judging from the results of their hydrolysis.

(9) Raw flesh which has been completely freed from proteids soluble in cold water contains two classes of proteid substances, those which are soluble in a 10 per cent. solution of ammonium sulphate and those which are insoluble in this medium.

(10) Of the total proteids of lean raw flesh about 16.00 per cent. is insoluble in cold water but soluble in a 10 per cent. solution of ammonium sulphate.

(11) A 10 per cent. ammonium sulphate extract of flesh contains at least two individual proteids or groups of proteids which differ in physical properties and to some extent in chemical composition; one of them however may possibly be identical with the meat residue not dissolved by the ammonium sulphate.

(12) Raw flesh which has been completely freed from proteids soluble in cold water and also from those soluble in a 10 per cent. solution of ammonium sulphate is almost entirely soluble in a N/20 solution of potassium hydroxide.

(13) The proteid material of flesh insoluble in water and in a 10 per cent. solution of ammonium sulphate, but soluble in a N/20 solution of potassium hydroxide, has the same chemical composition even when separated from the solvent by different means and purified by widely differing treatment.

URBANA, ILLINOIS,
February 12, 1906.

[CONTRIBUTION FROM THE LEATHER AND PAPER LABORATORY OF THE BUREAU OF CHEMISTRY.][1]

THE EXTRACTION OF TANNING MATERIALS FOR ANALYSIS.[2]

BY F. P. VEITCH AND H. H. HURT.

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AT THE last meeting of the Association of Official Agricultural Chemists, a paper was presented on "The Extraction of Tanning Materials with Various Extractors," which was published in part in this Journal.[3] It was shown that in the hands of the

[1] By permission of the Secretary of Agriculture.

[2] Read at the New Orleans Meeting of the American Chemical Society.

[3] Veitch: This Journal, 27, 724.

TABLE I.—RESULTS WITH DIFFERENT EXTRACTORS.

No.	Sample.	Total extract.		Soluble solids.		Reds.		Non-tannins.		Tannins.	
		Koch. Per. cent.	Continuous. Per cent.	Koch. Per cent.	Continuous. Per cent.	Koch. Per cent.	Continuous Per cent.	Koch. Per cent.	Continuous. Per cent.	Koch. Per cent.	Continuous. Per cent
"R"	Sumac, Sicily	....	51.22	44.53	47.94	....	....	20.02	20.93	24.51	27.01
75	Sumac, Sicily	40.64	48.88	38.39	45.13	2.25	3.65	17.88	21.02	20.25	24.11
76	Sumac, Sicily	41.58	54.42	39.80	48.86	1.78	5.56	22.30	24.01	17.50	24.85
80	Sumac, Sicily	50.14	58.37	48.16	56.34	1.98	1.93	21.82	27.10	26.34	29.24
437	Sumac, Sicily	53.87	60.13	49.35	59.97	4.52	0.16	17.65	24.05	31.70	35.92
132	Lentiscus, Sicily	37.65	45.06	34.89	39.13	2.76	5.93	19.23	22.73	15.66	16.40
69	Leaves A. manzanita, Calif.	40.65	50.24	39.75	48.17	0.90	2.07	25.41	32.21	14.34	15.96
314	Chestnut oak bark	18.30	20.84	16.86	19.27	1.44	1.57	6.85	7.58	10.01	11.69
316	Canaigre	52.91	55.30	46.73	48.75	6.18	6.55	15.20	15.96	31.73	32.79
315	Quebracho wood	26.13	28.55	24.40	27.14	1.73	1.41	2.38	2.72	22.02	24.34
385	Quebracho wood	28.93	32.68	24.84	27.90	4.09	4.78	....	3.41	....	24.49
	Quebracho wood	32.32[1]	31.63	28.12	27.43	4.20	4.20	3.96	2.98	24.16	24.45
386	Spent hemlock bark	9.30	10.94	8.41	9.04	0.89	1.90	4.28	3.98	4.13	5.06
438	New hemlock bark	16.47	23.35	15.53	20.99	0.84	2.36	4.64	7.31	10.89	13.58
781		63.12[2]	71.43	59.59	64.00	3.53	7.43	18.74	21.12	40.85	42.88
		65.54	71.78	60.96	64.76	4.58	7.02	21.68	20.78	39.28	43.98
	Chestnut wood	13.68[1]	12.50	12.28	11.20	1.40	1.30	4.75	3.63	7.43	7.57
		8.98	15.30[3]	8.53	13.83	0.40	4.47	2.25	4.88	5.28	8.95

[1] Weiss extractor. [2] Results by Krug. [3] Ground to 1 mm.

writer a continuous extractor such as the well known double tube, or that devised by Zulkowsky, gave more complete extraction and higher tannins than the Weiss or the Koch extractor, which are almost universally used in extracting tanning materials. In addition, this continuous extractor possesses the advantages of avoiding long boiling of the extract, undue concentration of the extract, and a saving in time required to concentrate dilute liquor. It has the further advantage of being more rapidly manipulated and is longer lived than some forms, while it is cheaper than other extractors that are used or have been proposed.

The results here reported are a continuation of the work then started, undertaken to determine if the continuous extractor gave different results from other extractors. For convenience, some of the results reported in the previous paper are included here, that the data on the commonly used material may be all together. All materials in the table were ground to pass through a 2 mm. mesh sieve.

It will be seen that without exception the results with the continuous extractor are higher than with any of the old forms in which the extraction was continued for a longer time than is customary. It was found necessary to continue the extractions with the Weiss extractor for from two to four days and the volume of extract was from 3 to 6 liters. It is possible of course that the long boiling, necessary to concentrate the last portions of these extracts, destroyed some tannin, a contingency which would have lowered the tannin figures by the Weiss method. But even under this long extraction all tannin was not removed from the materials, for upon transferring the residues to the continuous extractors a heavy precipitate of tannin was obtained in the extract, thus showing that tannin still remained in the material and indicating at the same time that the destruction of tannin by boiling had been small if any.

The results by the two methods on sumac and on canaigre are worthy of more than passing mention, as it has been held by many investigators that in extracting these materials the temperature must be kept at from 50° to 60° C. until at least half the volume of the extract is obtained, when it is permissible to raise the temperature. With the continuous extractor it is our

custom to extract with from 200 to 300 cc. of water below 100° C. and finish the extraction at steam heat. It seems to the writers that the reasons advanced for the extraction of certain materials at low temperature are not good ones. If all the tannin is not removed in the extract obtained at low temperature, there is every reason to believe that the gelatinization of the starch at high temperatures would prevent the extraction of the remaining tannin or possibly precipitate a portion of the extracted tannin. That this is not so, is evidenced by the results with the continuous extractor and also by the fact that even with the old form of extractor much tannin is removed on increasing the heat. However, speculation on these points is of little value as it is probable that complete extraction of any material by the older methods, except by the use of the Soxhlet, has seldom been obtained and was certainly not obtained in these experiments. We are convinced, however, that a Soxhlet which does not require too great concentration of the extracted material between the siphonings will give complete extraction and higher tannin; but with so large a charge as is generally used in this work the Soxhlet is likely to become continuous in operation. Undue concentration of the liquors is best prevented by the removal of the liquors and their replacement by water after the extraction has proceeded but a short time.

No indication as to the condensation of tannins to reds can be obtained from the figures given, as in nearly all cases where the continuous extractor was used some finely ground material passed into the extract. In some cases, however, the reds are as low as with the Koch extractor, indicating no greater change in these cases than with the Koch extractor. This is true with sumac, with chestnut oak bark, with quebracho wood and with canaigre, the last three of which, being representative of catechol tannins, would be most likely to show such change.

As a further test of various methods of extracting, samples of sumac and of oak bark which were ground to pass a 2 mm. mesh were extracted in several ways.

(1) By the Proctor extractor, beginning the extraction at 50° and gradually raising the temperature to 100°, the extraction being discontinued when 1 liter was reached, at seven hours.

(2) By the continuous extractor for the same time.

(3) By the continuous extractor for sixteen hours.

(4) By the Soxhlet extractor for sixteen hours, removing the extracted material as in the continuous method.

The results are given in Table II.

TABLE II.—EXTRACTION OF SUMAC AND OAK BARK BY DIFFERENT METHODS.

Material.	Method of extraction.	Total extract.	Soluble solids.	Reds.	Non-tannin.	Tannin.
Sumac No. 420	Continuous, 16 hours.....	57.00	53.85	3.15	19.80[1]	34.05
Sumac No. 420	Continuous, 7 hours.....	53.45	51.27	2.18	17.97[1]	33.30
Sumac No. 420	Siphon Soxhlet, 16 hours .	54.69	51.63	2.06	17.53[1]	34.10
Sumac No. 420	Proctor, 7 hours to 1 liter.	48.33	46.78	1.54	16.60[1]	30.19
Oak bark No. 314	Continuous, 16 hours.....	21.69	19.76	1.93	7.96[2]	11.80
Oak bark No. 314	Continuous, 7 hours.....	19.23	17.63	1.60	6.65[1]	10.98
Oak bark No. 314	Siphon Soxhlet, 16 hours .	19.57	18.29	1.28	7.09[1]	11.20
Oak bark No. 314	Proctor, 7 hours to 1 liter.	15.25	14.91	0.34	5.77[1]	9.14

With these materials the continuous extractor for sixteen hours and the siphon extractor (which in reality acted as a continuous extractor) gave practically the same results on tannin. The continuous extractor that was run for the same length of time that was required to collect 1 liter with the Proctor extractor gave slightly lower results, while the Proctor extractor, extracting but 1 liter, gave the lowest results, and in both cases the extract then coming through was colored and gave a heavy precipitate with gelatine solution. It is quite evident that by the latter method extraction was far from complete and that decidedly better results are obtained with the continuous extractor run for the same length of time.

The sample marked "R" furnishes much evidence on the difficulty of completely extracting tanning materials by the accepted procedure. This sample was submitted to the senior author in 1901 for analysis and gave the figures given under the "Koch" heading in Table I. The results by the continuous extractor were secured recently by Mr. Hurt. At the time the sample was submitted in 1901 it was also analyzed by another chemist, using the Proctor or Weiss form of extractor, who reported total extract 39.86 per cent., soluble solids 35.98 per cent., non-tannins 18.34 per cent. and tannin 17.64 per cent., showing that the extraction was far from complete. To secure more light on extraction, another sample of sumac was treated as follows, using the Koch extractor for the work. In all experi-

[1] No tannin in filtrate.

[2] Tannin in filtrate.

ments, the first 500 cc. of percolate were secured in from one and one-quarter to two hours at from 60° to 70° C. The balance secured at steam heat was concentrated to the desired volume.

Experiment I.—Twenty grams extracted, total percolate 3½ liters. After last half liter, percolate still gave slight opacity with gelatine solution.

	Per cent.
Total extract	48.72
Soluble solids	47.50
Non-tannins	20.38
Available tannin	27.12

Experiment II.—Twenty grams extracted, percolate 4 liters, concentrated to 1 liter. No apparent opacity with gelatine after the last ½ liter of percolate, but still gave a distinct color with ferric sulphate.

	Per cent.
Total extract	51.95
Soluble solids	47.10
Non-tannins	17.90
Available tannin	29.20

Experiment III.—Twenty grams extracted with 2 liters of water, the last 1500 cc. being boiled down to 500 cc. and added to the first 500 cc. Time of extraction about seven hours.

	Per cent.
Total extract	45.67
Soluble solids	43.10
Non-tannins	16.76
Available tannin	26.34

Percolate after the last ½ liter showed tannin with gelatine.

Experiment IV.—Continued the extraction of the 20 grams used in Expt. III, percolated 2 liters at temperature of boiling water, after which the test with gelatine was uncertain, but color was strong with ferric sulphate. Boiled down the 2 liters to ½ liter and made analysis. Time of extraction about three hours.

	Per cent.
Total extract	5.00
Soluble solids	3.58
Non-tannins	1.76
Available tannin	1.82

Experiment V.—The percolation of the same 20 grams sample used in Expts. III and IV was continued until 4 liters more water had been percolated, when the percolate was boiled down to ½

liter and analyzed as in Expts. III and IV. Time of extraction about six hours.

	Per cent.
Total solids	3.37
Soluble solids	2.39
Non-tannins	1.03
Available tannin	1.36

Adding results of Expts. III, IV and V we have a total of

	Per cent.
Total extract	54.04
Soluble solids	49.07
Non-tannins	19.55
Available tannin	29.52

extracted by 8 liters of water at about 100° C. in about sixteen hours. The residues from these extractions were dried and amounted to 37.20 per cent.

	Per cent.
Residue	37.20
Moisture in sample	7.93
Total extract	54.04
	99.17
Amount of sample lost in analysis	0.83.

These results show that extraction of tannin was not complete with percolation of $3\frac{1}{2}$ liters of water, but was practically complete with 4 liters and that 8 liters removed but little more tannin but did remove more soluble non-tannins.

EFFECT OF FINENESS OF MATERIAL.

The size of the particles of the material to be extracted will certainly influence the time required for complete extraction. Some data on this point are shown in Table III.

Time of extraction about eighteen hours.

TABLE III.—RESULTS ON MATERIALS OF VARYING FINENESS.

Fineness of sample.	Total extract. Per cent.	Soluble solids. Per cent.	Non-tannins. Per cent.	Tannin. Per cent.
Quebracho wood ground to 4 mm. mesh	29.30	26.24	3.17	23.07
Quebracho wood ground to 2 mm. mesh	32.68	27.90	3.41	24.49
Quebracho wood ground to 1 mm. mesh	31.14	27.43	3.31	24.12
Spent hemlock bark ground to 2 mm. mesh	11.10	....	4.20	....
Spent hemlock bark ground to 1 mm. mesh	10.94	9.04	3.98	5.06
Chestnut wood ground to 4 mm. mesh	15.30	13.83	4.88	8.95
Chestnut wood ground to 1 mm. mesh	16.07	13.81	4.96	8.84

In all cases a trace of tannin could be detected on further extracting the materials.

The results given in this paper confirm the conclusions previously drawn that the continuous extractor has given the most complete extraction and that by this apparatus all soluble constituents are removed in larger quantities than by other extractors. It may be said, however, that while we believe this to be the best extractor with which we are acquainted, it is not to be doubted that other forms can be made to give as complete extraction, but at a greater expenditure of money, time, and labor.

We desire especially to call attention to the necessity for complete extraction. With the comparatively large quantity of material that is used it is exceedingly difficult to secure this. All materials should be ground to pass at most a millimeter sieve, and extraction should be continued for at least sixteen hours, preferably for twenty-four hours. The efforts of this laboratory are now being directed toward so modifying the methods of tannin analysis that smaller quantities of material may be used and thus secure more complete extraction.

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THE EXAMINATION OF WRITING INKS.[1]

BY L. S. MUNSON.

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IN many branches of the government service as well as in many state and private institutions it is frequently of particular importance that permanent records be preserved, and from necessity or convenience many of these are written records. It is well known that many of the old writing fluids yielded records that have remained unchanged for generations, and undoubtedly many of the writing inks in use at the present time are equally resistant. However, from the great advances made in recent years in the manufacture of colors has arisen the tendency to substitute these colors in whole or in part in the manufacture of ink. This investigation was taken up, therefore, to determine to what extent the inks in use in the different executive departments were suitable for record purposes.

[1] Read at the New Orleans Meeting of the American Chemical Society.

A total of thirty samples was examined. These represented eighteen distinct brands of ink and were the products of eight manufacturers. Three of these samples were for use expressly as copying inks, three were combined writing and copying inks, and of the remaining twenty-four the claim was made with eight that they would furnish good copies, especially when fresh. Twenty-seven of the samples were iron-tannin inks and three were chromate-logwood inks. There are but few of the latter class of inks upon the market, so this work had to do principally with the iron-tannin inks. These latter depend upon the tannate of iron which they contain for their permanence, and the principal ingredients entering into their composition are tannic acid, or a mixture of tannic and gallic acids, ferrous sulphate, a small amount of acid to keep the tannate of iron in solution, sufficient soluble dye to give the immediate color to the ink, and a small amount of preservative to prevent decomposition or the development of moulds. With such an ink the full development of the color takes place only after exposure of the writing to the air for a period of several days, hence the necessity of a considerable amount of soluble dye to give the immediate color. In case the ink is intended for copying purposes some hygroscopic material is added to facilitate this purpose.

The work on these inks included (1) chemical analysis of the ink; (2) the effect of exposure to sunlight and of treatment with reagents upon stripes made with the ink; and (3) the nature of the copy furnished, the effect upon this copy of exposure to sunlight, and the effect upon the original writing of taking press copies from the same. Full results of analysis and of the various tests applied are given in the accompanying table. Samples Nos. 1851, 1965 and 1986 were copying inks, Nos. 1720, 1964 and 1985 were combined writing and copying inks, and of the remaining samples the claim was made for Nos. 1962, 1330, 1717, 1961, 1722, 1329, 1727 and 1732 that they would yield good copies from the fresh writing.

Discussion of Results.—The specific gravity, total solids, ash, iron and sulphates were determined on all the samples except the logwood inks, in which cases instead of iron, potassium dichromate was determined. Of these, specific gravity and iron are considered of particular importance in determining the quality of

an ink. The specific gravity of the copying inks ranged from 1.0634 to 1.1093, of the combined writing and copying inks from 1.0473 to 1.0493, and of the writing inks from 1.0068 to 1.0411. The percentage of iron ranged in the copying inks from 1.46 to 1.80, in the combined writing and copying inks from 0.73 to 0.80, and in the writing inks from 0.05 to 0.68. Of the copying inks, but two were iron-tannin inks, so the range given above for iron is not to be considered representative of this class of inks. In fact, the two iron-tannin inks examined were particularly heavy inks. One copying ink was a chromate-logwood ink containing 0.06 per cent. of potassium dichromate, and two writing inks were chromate-logwood inks containing 0.73 and 0.74 per cent. of potassium dichromate.

It is seen from the above that there are distinct differences in composition between the various classes of ink, and wide ranges between inks of the same class. With many writing inks permanence is not an essential feature, and in such cases the lighter and cheaper inks may serve the purpose as well as the heavier and more expensive ones. The results obtained in this investigation enable one to distinguish those inks that may be relied upon to produce permanent records, from those the writings of which are not permanent. By a permanent ink is meant one the writing of which, when exposed to the usual conditions of moderate heat, atmospheric moisture and sunlight, will resist destruction. It is well known that the iron-tannin inks as well as the chromate-logwood inks are easily destroyed by various chemical agents, notably acids, oxidizing agents, etc.

Upon exposure to sunlight and treatment with reagents the various inks showed marked differences in behavior. For these tests stripes were made upon good quality of paper by allowing the ink to flow from a pipette inclined at an angle of 45° with the paper, using (1) the undiluted ink and (2) the ink diluted with an equal volume of water. Upon exposure of the stripes to sunlight, valuable information was obtained regarding the lasting qualities of the inks. When the stripes made with the undiluted ink were exposed for a period of twenty days a number of the lighter inks showed marked deterioration, and when the time of exposure was increased to eighty days all the iron-tannin inks containing less than 0.30 per cent. of iron, and all the chromate-logwood inks were badly faded, while a number of the iron-tannin inks

Serial number.	Specific gravity. 15.5° C.	Total solids. Per cent.	Ash. Per cent.	Iron. Per cent.	Sulphates as SO_3. Per cent.	Exposed 20 days.
1721	1.0273	4.13	1.23	0.37	0.98	No change.
1960	1.0264	4.14	1.08	0.35	0.65	No change.
1962	1.0207	3.22	0.62	0.38	0.80	No change.
1330	1.0205	3.06	0.61	0.33	0.75	No change.
1717	1.0207	3.28	0.62	0.32	0.73	No change.
1718	1.0395	6.50	1.17	0.65	1.06	No change.
1850	1.0363	5.95	1.08	0.60	0.94	No change.
1982	1.0367	6.04	1.05	0.67	0.97	No change.
1720	1.0473	7.63	1.39	0.73	1.61	No change.
1964	1.0473	7.43	1.40	0.80	1.55	No change.
1985	1.0493	7.95	1.41	0.79	1.54	No change.
1851	1.0887	13.84	2.65	1.46	2.38	No change.
1986	1.1093	16.87	3.01	1.80	3.25	No change.
1965	1.0634	11.20	3.91	0.06 $K_2Cr_2O_7$	0.97	Color changed to red.
1432	1.0069	1.36	0.18	0.05	0.28	Faded badly.
1433	1.0209	3.48	0.67	0.26	0.79	No change.
1434	1.0182	2.81	0.50	0.27	0.71	No change.
1981	1.0068	1.26	0.17	0.14	0.25	Faded badly.
1961	1.0377	6.21	1.20	0.68	1.07	No change.
1729	1.0175	2.71	0.58	0.23	0.58	Faded badly.
1984	1.0204	3.17	0.59	0.31	0.70	No change.
1722	1.0169	3.01	0.85	0.73 $K_2Cr_2O_7$	0.02	Faded slightly.
1725	1.0169	3.00	0.86	0.74 $K_2Cr_2O_7$	0.03	Faded slightly.
1329	1.0185	3.36	0.85	0.14	0.38	No change.
1727	1.0411	7.15	1.18	0.50	0.82	No change.
1732	1.0160	2.76	0.80	0.16	0.38	Faded slightly.
1963	1.0204	3.42	0.88	0.19	0.46	No change.
1723	1.0120	1.97	0.55	0.14	0.27	No change.
1730	1.0099	1.72	0.45	0.12	0.24	Faded slightly.
1431	1.0377	7.43	0.88	0.32	0.58	No change.

Sulphates as SO_3. Per cent.	Exposed to sunlight. 20 days.	80 days.	
0.98	No change.	Faded badly.	Li
0.65	No change.	Faded slightly.	Ba
0.80	No change.	No change.	Ba
0.75	No change.	Faded badly.	Li
0.73	No change.	No change.	Li
1.06	No change.	No change.	Li
0.94	No change.	No change.	Li
0.97	No change.	No change.	Li
1.61	No change.	No change.	Li
1.55	No change.	No change.	Li
1.54	No change.	No change.	Li
2.38	No change.	No change.	Li
3.25	No change.	No change.	Li
O_7 0.97	Color changed to red.	Color changed to red.	Al
0.28	Faded badly.	Faded almost completely.	Ba
0.79	No change.	Faded badly.	Li
0.71	No change.	Faded badly.	Li
0.25	Faded badly.	Faded very badly.	De
1.07	No change.	No change.	Li
0.58	Faded badly.	Faded very badly.	Ba
0.70	No change.	Faded slightly.	Ba
O_7 0.02	Faded slightly.	Faded very badly.	Ba
O_7 0.03	Faded slightly.	Faded very badly.	Ba
0.38	No change.	Faded badly.	Ba
0.82	No change.	Faded badly.	Li
0.38	Faded slightly.	Faded very badly.	Ba
0.46	No change.	Faded badly.	Al
0.27	No change.	Faded badly.	Ba
0.24	Faded slightly.	Faded very badly.	Ba
0.58	No change.	No change.	Li

	Test of stripes made with ink.				
	A—undiluted.				B—diluted wi Exp
	Treated with N/200 $Ca(OCl)_2$.		Treated with water 3 days.	Treated with 95 per cent. alcohol. 3 days.	20 days.
	15 minutes.	1 hour.			
	Little affected.	Destroyed.	No change.	No change.	No change.
	Badly affected.	Badly affected.	Faded slightly.	No change.	No change.
	Badly affected.	Destroyed.	Faded slightly.	No change.	No change.
	Little affected.	Destroyed.	No change.	No change.	Faded slightly.
	Little affected.	Destroyed.	No change.	No change.	No change.
	Little affected.	Least affected.	No change.	No change.	No change.
	Little affected.	Badly affected.	No change.	No change.	No change.
	Little affected.	Least affected.	No change.	No change.	No change.
	Little affected.	Badly affected.	No change.	No change.	No change.
	Little affected.	Least affected.	No change.	No change.	No change.
	Little affected.	Least affected.	No change.	No change.	No change.
	Little affected.	Least affected.	No change.	No change.	No change.
	Little affected.	Least affected.	No change.	No change.	No change.
o red.	Almost destroyed.	Destroyed.	Faded badly.	No change.	Color changed to
npletely.	Badly affected.	Destroyed.	No change.	No change.	Faded badly.
	Little affected.	Badly affected.	No change.	No change.	Faded slightly.
	Little affected.	Destroyed.	No change.	No change.	Faded slightly.
y.	Destroyed.	———	Faded slightly.	Faded slightly.	Faded badly.
	Little affected.	Least affected.	No change.	No change.	No change.
y.	Badly affected.	Destroyed.	Faded slightly.	No change.	Faded badly.
	Badly affected.	Badly affected.	Faded slightly.	No change.	Faded slightly.
r.	Badly affected.	Destroyed.	No change.	No change.	Faded badly.
r.	Badly affected.	Destroyed.	No change.	No change.	Faded badly.
	Badly affected.	Destroyed.	Faded badly.	No change.	Faded badly.
	Little affected.	Destroyed.	No change.	No change.	No change.
y.	Badly affected.	Destroyed.	Faded slightly.	No change.	Faded badly.
	Almost destroyed.	Destroyed.	Faded badly.	No change.	Faded slightly.
	Badly affected.	Destroyed.	Faded badly.	Faded slightly.	Faded badly.
.	Badly affected.	Destroyed.	Faded badly.	Faded slightly.	Faded badly.
	Little affected.	Least affected.	No change.	No change.	No change.

Test of stripes made with ink.

Treated with water 3 days.	Treated with 95 per cent. alcohol. 3 days.	B—diluted with equal volume of water. Exposed to sunlight. 20 days.	80 days.	Nature of copy furnished. A. With fresh writing.	With writing 2 days old.	Effect of exposure of copy. Copy A. 20 days.	80 days.	
o change.	No change.	No change.	Faded very badly.					
aded slightly.	No change.	No change.	Faded badly.					
aded slightly.	No change.	No change.	Faded badly.	Poor.	Poor.	Faded badly.	Faded very badly.	Faded
o change.	No change.	Faded slightly.	Faded very badly.	Fair.	Fair.	Faded almost completely.	Faded completely.	Faded
o change.	No change.	No change.	Faded badly.	Good.	Fair.	Faded almost completely.	Faded completely.	Faded
o change.	No change.	No change.	No change.	Very good.	Very good.	Faded badly.	Faded almost completely.	Faded
o change.	No change.	No change.	No change.	Good.	Good.	Faded badly.	Faded badly.	Faded
o change.	No change.	No change.	Faded badly.	Good.	Good.	Faded badly.	Faded very badly.	Faded
o change.	No change.	No change.	No change.	Very good.	Very good.	Faded badly.	Faded very badly.	Faded
o change.	No change.	No change.	No change.	Good.	Good.	Faded badly.	Faded badly.	Faded
o change.	No change.	No change.	No change.	Good.	Good.	Faded badly.	Faded badly.	Faded
o change.	No change.	No change.	No change.	Very good.	Very good.	Faded slightly.	Still good copy.	Faded
o change.	No change.	No change.	No change.	Very good.	Very good.	Faded slightly.	Still good copy.	Faded
aded badly.	No change.	Color changed to red.	Faded badly.	Good.	Good.	Faded badly.	Faded very badly.	Faded
o change.	No change.	Faded badly.	Faded completely.					
o change.	No change.	Faded slightly.	Faded very badly.					
o change.	No change.	Faded slightly.	Faded very badly.					
aded slightly.	Faded slightly.	Faded badly.	Faded completely.					
o change.	No change.	No change.	Faded slightly.	Fair.	Fair.	Faded badly.	Faded badly.	Faded
aded slightly.	No change.	Faded badly.	Faded completely.					
aded slightly.	No change.	Faded slightly.	Faded badly.					
o change.	No change.	Faded badly.	Faded almost completely.	Poor.	Poor.	Faded almost completely.	Faded completely.	Faded
o change.	No change.	Faded badly.	Faded almost completely.					
aded badly.	No change.	Faded badly.	Faded very badly.	Fair.	Fair.	Faded almost completely.	Faded completely.	Faded
o change.	No change.	No change.	Faded very badly.	Good.	Good.	Faded very badly.	Faded completely.	Faded
aded slightly.	No change.	Faded badly.	Faded almost completely.	Fair.	Poor.	Faded almost completely.	Faded completely.	Faded
aded badly.	No change.	Faded slightly.	Faded very badly.					
aded badly.	Faded slightly.	Faded badly.	Faded almost completely.					
aded badly.	Faded slightly.	Faded badly.	Faded completely.					
o change.	No change.	No change.	No change.					

posure of copy to sunlight.		Exposure of writing to sunlight.	
Copy B.			
20 days.	80 days.	Original writing.	Writing after press-copying.
Faded very badly.	Faded almost completely.	No change.	Faded very badly.
Faded almost completely.	Faded completely.	No change.	Faded badly.
Faded almost completely.	Faded completely.	Faded slightly.	Faded badly.
etely. Faded badly.	Faded almost completely.	No change.	Faded slightly.
Faded very badly.	Faded completely.	No change.	Faded very badly.
Faded badly.	Faded very badly.	No change.	Faded badly.
Faded badly.	Faded very badly.	No change.	Faded slightly.
Faded badly.	Faded badly.	No change.	Faded badly.
Faded badly.	Faded badly.	No change.	Faded slightly.
Faded slightly.	Still good copy.	No change.	Faded slightly.
Faded slightly.	Still good copy.	No change.	No change.
Faded badly.	Faded almost completely.	No change.	Faded almost completely.
Faded badly.	Faded badly.	No change.	Faded badly.
Faded completely.	———	Faded slightly.	Faded badly.
Faded completely.	———	Faded badly.	Faded almost completely.
Faded very badly.	Faded completely.	No change.	Faded very badly.
Faded completely.	———	Faded badly.	Faded badly.

containing more than 0.30 per cent. of iron showed marked deterioration. Stripes made with the diluted ink were much more readily affected, but a number remained unaffected after eighty days' exposure. Samples Nos. 1718, 1850, 1720, 1964, 1985, 1851, 1986, 1961 and 1431 showed little or no change when submitted to this test and are the only inks that are considered entirely satisfactory for record purposes.

When treated with calcium hypochlorite of a strength corresponding to N/200 chlorine the color of the ink was entirely destroyed in all cases, the length of time required depending largely upon the quality of the ink. In a number of cases, notably with the lighter inks, the color was entirely destroyed in less than fifteen minutes, while with the heaviest inks the time required was from one to two hours. This test, of course, gives only comparative results, but is of value as a ready means of determining the relative values of a number of inks.

Upon treating the stripes with water and with 95 per cent. alcohol a number of the inks showed marked loss of color, notably those containing small amounts of tannate of iron, and depending largely for their coloring power upon the soluble dye present in them. A good ink should undergo practically no change upon treating with water or with alcohol.

For the purpose of studying the nature and permanence of the copy furnished by the different inks, one copy was taken from the fresh writing and one copy from writing two days old. In nearly all cases the copy obtained from the writing two days old was as good as that taken from the fresh writing, but to obtain such a copy it was necessary to use somewhat moister pads and to leave the copy in press longer than with fresh writing. Only the heavy copying inks gave copies that were at all permanent when exposed to sunlight. With many the copy faded almost completely upon twenty days' exposure. This rapid fading of the copy is due in part to the small amount of ink that is actually transferred to and retained by the copy. While the writing may, and generally does, give up a considerable quantity of ink when the copy is taken, much of this passes into the moist pad and but little is actually retained by the light, porous paper upon which the copy is generally taken. Besides, an ink may readily give up the soluble dye, while but little tannate of iron is trans-

ferred to the copy. In such case the power of resistance of the copy is very low.

The writing after press copying was exposed to sunlight along with the writing from which no press copy had been taken. In nearly all cases the former faded much more readily, and in some cases the difference was exceedingly marked, the greatest difference being found in the lighter inks.

The inks examined represent the products of nearly all the large manufacturers in this country and show a very wide range in composition. Many of these are unsuited for record purposes while several are entirely satisfactory as record inks.

The United States Treasury Department requires that the ink furnished that department shall equal in quality an ink made up with 23.4 grams tannic acid, 7.7 grams gallic acid, 30 grams ferrous sulphate, 10 grams gum arabic, 25 cc. dilute hydrochloric acid and 1 gram carbolic acid, the whole being made up in volume to 1000 cc. A number of the inks examined were apparently made up to meet the above requirement. Such an ink would have a specific gravity of about 1.0360 and contain about 0.60 per cent. of iron, and would meet all ordinary requirement of a record ink. In fact, a few inks that were somewhat lighter than the above were found entirely satisfactory for record purposes. It is well, however, to have the requirements of such an ink amply high so long as its working qualities are not interfered with.

CONTRACTS LABORATORY.

NITROSYL SELENIC ACID.

BY VICTOR LENHER AND J. H. MATHEWS.

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NITROGEN tetroxide, when brought in contact with sulphuric acid, yields the well-known nitrosyl sulphuric acid. When selenic acid is substituted for the sulphuric acid, a product is obtained analogous to nitrosyl sulphuric acid. However, in the case of the preparation of nitrosyl selenic acid, it is necessary that a low temperature should be used.

When selenic acid having a strength of 83 per cent. is mixed with an excess of liquid nitrogen tetroxide and the solution cooled with solid carbon dioxide, a light blue solid forms on the surface, and immediately below this a layer of a darker blue solid. These two layers form over a heavy syrupy liquid, consisting

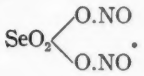
mainly of selenic acid. The light blue superficial layer, when submitted to analysis, shows a high content of nitrogen, but repeated analyses have shown this substance to be a mixture of varying amounts of the excess of nitrogen tetroxide with the material composing the darker blue layer. The two layers are defined so sharply that the dark blue material can be easily obtained in pure condition.

This blue solid can also be formed by using a more concentrated selenic acid, adding the nitrogen tetroxide and introducing a small quantity of water; considerable variation in the strength of the acid does not materially affect the formation of the two layers of light and darker blue solid, nor their composition.

The dark blue solid forms and melts at —13°. It fumes strongly in the air with the liberation of the oxide of nitrogen and selenic acid remains.

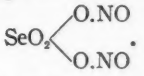
Analysis: 0.1120 gram substance gave 0.0333 gram nitric oxide equivalent to 29.7 per cent., and 0.1131 gram of substance gave 0.03417 gram nitric oxide corresponding to 30.2 per cent.

If both of the hydroxyl groups in selenic acid be replaced by the —O.NO group we have

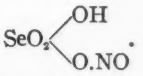


This compound requires 29.55 per cent. of nitric oxide. The analytical results obtained from the material composing the blue layer accord with this theoretical amount.

It thus appears that when nitrogen tetroxide acts upon fairly strong selenic acid at low temperatures it is possible to replace both of the hydroxyl groups and obtain the body



Although many products were examined, we have not been able to prepare the true nitrosyl selenic acid having the composition



The compound described is of constant composition. It is decomposed by water in a manner analogous to nitrosyl sulphuric acid. It is not stable above —13°, losing the oxide of nitrogen

and reverting to selenic acid. All of the methods which are described in the literature for the preparation of nitrosyl sulphuric acid have been tried with selenic acid but all others than the method described have proven fruitless in our hands.

CHEMICAL LABORATORY, UNIVERSITY OF WISCONSIN.

MONOHALOGEN DERIVATIVES OF TRIPHENYLCARBINOL CHLORIDE.

BY L. H. CONE AND C. P. LONG.

Received February 10, 1906.

IT WAS shown by Gomberg[1] that the monohalogen derivatives of triphenylcarbinol chloride can be prepared from benzophenone dichloride and the corresponding phenyl halide by the Friedel and Crafts reaction. The position of the halogen was assumed to be the para. This was proven in the case of the monochlor compound by preparing it from the dichloride of *p*-chlorbenzophenone and benzene. We now undertook to condense in a similar way the dichloride from *p*-brombenzophenone with benzene in order to determine the position of the bromine in the monobrom compound. Very unexpected results were obtained. They were found to be due to an abnormal reaction of phosphorus pentachloride with the *p*-brombenzophenone. Recourse was had to Grignard's reaction, using both the methyl ester of *p*-brombenzoic acid and *p*-brombenzophenone as starting materials. The monobromtriphenylcarbinol chlorides from both of these materials were identical, and were, of necessity, the para-compound. The comparison of the carbinol chloride from the benzophenone dichloride and brombenzene by the Friedel and Crafts reaction with that obtained by Grignard's reaction showed the former to be also the *p*-compound.

1. *Preparation of p-Bromtriphenylcarbinol Chloride by the Friedel and Crafts Reaction,* (p-$BrC_6H_4(C_6H_5)_2CCl$).[1]—A mixture of an excess of brombenzene (2-3 mol.) and benzophenone dichloride (1 mol.) was treated with a small quantity of aluminum chloride and heated until the reaction started. It was usually necessary to heat the mixture to about 115°. After the reaction was well started, the temperature was allowed to drop to 80°. The reaction was maintained smoothly at this temperature for several hours by frequent additions of aluminum chloride. The reaction

[1] Ber. 37, 1633.

mixture was decomposed with ice and hydrochloric acid. After removing the excess of brombenzene by steam distillation, the oily layer was taken up in benzene and dried over calcium chloride. The benzene solution was saturated in the presence of solid calcium chloride with dry hydrochloric acid in order to convert the carbinol to the carbinol chloride. The benzene was distilled off, the last portions under diminished pressure. The residue was taken up in petroleum ether (70–80°) and purified with animal charcoal. Transparent, prismatic crystals of melting-point 111° C. separated on standing. Yield 55 per cent. of the theoretical.

2. *Preparation by Grignard's Reaction.*—The carbinol obtained by Grignard's reaction from *p*-brombenzoic methyl ester and the magnesium compound of brombenzene was changed into the carbinol chloride by dry hydrochloric acid gas. The purified product had a melting-point of 113.5°. The same carbinol chloride was also obtained by Grignard's reaction from *p*-brombenzophenone and the magnesium compound of brombenzene. The melting-point of this product was 114°. This compound was shown to be identical with the product of the Friedel and Crafts reaction by a series of derivatives.

p-Bromtriphenylcarbinol ($p\text{-}BrC_6H_4(C_6H_5)_2COH$). — The carbinol was prepared by dissolving 3 grams of the carbinol chloride in a mixture of glacial acetic and sulphuric acids (3 : 1)[1] with gentle heating to remove the hydrochloric acid liberated. The acid solution was poured with stirring into a large volume of water. The carbinol separated as a white flocculent precipitate and was extracted with ether. The ethereal extract was washed with sodium carbonate and then dried over fused sodium sulphate. After distillation of the ether, the residue was taken up in petroleum ether. For several weeks the carbinol showed no tendency to separate except as an oil, but on longer standing, it crystallized. After several crystallizations it melted at 74°.

p-Bromtriphenylmethyl Peroxide ($p\text{-}BrC_6H_4(C_6H_5)_2CO)_2$.—The peroxide was prepared from the *p*-bromtriphenylcarbinol chloride by shaking a benzene solution of the latter with a solution of sodium peroxide.[2] The residue left by evaporation of the benzene solution was washed with ether, giving a peroxide melting at

[1] Am. Ch. J. 25, 328.

[2] Ber. 33, 3155; 37, 1630.

167°. The peroxide is slightly soluble in ether and also in petroleum ether. Small quantities of the peroxide were recrystallized from both of these solvents. Melting-point 171.5–173.5°.

The analyses by Carius' method gave 22.69 and 23.52 per cent. Br. Calculated, 23.66 per cent.

p-Bromtriphenylmethylamine (p-$BrC_6H_4(C_6H_5)_2CNH_2$).[1] — The amide was formed by passing dry ammonia into a benzene solution of the carbinol chloride, filtering out the precipitate of ammonium chloride, and taking up the residue, left by distillation of the benzene, in petroleum ether. The crystals formed melted at 108.5–109°.

p-Bromtriphenylmethylaniline (p-$BrC_6H_4(C_6H_5)_2CNHC_6H_5$).[2] — A benzene solution of one molecule of p-bromcarbinol chloride was treated with two and a half molecules of freshly distilled aniline. The oil, left after the removal of the aniline hydrochloride and the benzene, was taken up in ether, in which it is rather insoluble. Crystals formed on concentrating the solution. These were carefully washed with ether to remove as much as possible the excess of aniline and then crystallized from petroleum ether, melting-point 148°.

An analysis gave the following results:

Calculated for p-$BrC_6H_4(C_6H_5)_2CNHC_6H_5$: N, 3.39 per cent. Found: N, 3.63 and 3.88 per cent.

3. *Action of Phosphorus Pentachloride on p-Brombenzophenone.*— As mentioned above, unexpected results were obtained in attempting to prepare p-monobromtriphenylcarbinol chloride by the action of benzene on the dichloride from p-brombenzophenone. These were finally found to be due to the abnormal action of phosphorus pentachloride on the p-brombenzophenone used in making the dichloride.

p-Brombenzophenone (p-$BrC_6H_4COC_6H_5$).[3]—Kollaritz and Merz[4] obtained a monobrombenzophenone of a melting-point of 81.5° by heating benzoic acid and brombenzene with phosphorus

[1] Ber., 35, 1827.

[2] Ibid. 35, 1829.

[3] Richter in the "Lexikon der Kohlenstoffverbindungen," Supplement II, p. 255, gives an incorrect value (172°–173°) for the melting-point of p-brombenzophenone, and in Vol. II, p. 1302, gives Schafer's value (81.5°) for p-brombenzophenone as that of the meta compound.

[4] Ber. 6, 547.

pentoxide. Schafer[1] prepared it from *p*-brombenzoyl chloride and benzene by the Friedel and Crafts reaction. It can, however, be prepared much more easily and from cheaper materials by a method used in this laboratory for the preparation of *p*-chlorbenzophenone with nearly quantitative results. To a mixture of an excess of brombenzene (2-3 mol.) and benzoyl chloride (1 mol.) heated to 115°, some aluminum chloride was added to start the reaction. The temperature was then lowered to 80° as in the preparation of *p*-bromtriphenylcarbinol chloride. The reaction was maintained at this temperature for several hours with additions of small quantities of aluminum chloride. The reaction mixture was treated with ice and hydrochloric acid. The excess of brombenzene was removed by steam distillation. The light brown oily layer solidified on cooling. It was taken up in carbon disulphide, washed with sodium carbonate to remove any benzoic acid, and then dried. The solvent was evaporated and the residue purified by distillation *in vacuo*. The brombenzophenone distilled at 210° under 19–20 mm. as a colorless liquid which solidified to a pure white solid. The smallest yield was 65 per cent. while yields of 75–80 per cent. were usually obtained. The product is rather soluble in benzene and ether, and less soluble in petroleum ether and alcohol. It crystallizes from the last two solvents as needles, melting at 81.5–82°. A sample which melted at 81.5° was prepared from *p*-brombenzoyl chloride and benzene according to Schafer.[2]

Action of Phosphorus Pentachloride.—The *p*-brombenzophenone was mixed with 1.3 times the calculated quantity of phosphorus pentachloride required for the formation of a phenone dichloride. The mixture was heated in an oil-bath at 150° for five hours. The excess of phosphorus penta- and oxychlorides was removed and the residue distilled under diminished pressure. It distilled at 212–220° under 19 mm. This dichloride, assumed at first to be the pure parabrom compound, was mixed with several molecules of benzene and condensed by aluminum chloride at 90°. The carbinol chloride obtained on working up the reaction product crystallized as prismatic crystals very similar to the expected *p*-bromtriphenylcarbinol chloride. The melting-point of these was 95.5° and could be brought up finally as high as 97.5–98°,

[1] Ann. 264, 152.

[2] Loc. cit.

while that of *p*-bromtriphenylcarbinol chloride from Grignard's reaction was 113.5° and from benzophenone dichloride and brombenzene by the Friedel and Crafts reaction was 111°. The carbinol obtained from the product crystallized readily in contrast to the *p*-bromtriphenylcarbinol and had a melting-point of 81.5° and, after many repeated recrystallizations, of 83.5°, while the *p*-bromtriphenylcarbinol from the carbinol chloride of Grignard's reaction melted at 74° and *p*-chlortriphenylcarbinol at 85°.

Analyses of the carbinol chloride for chlorine which could be obtained by hydrolysis, gave results which were much too high for a monobromtriphenylcarbinol chloride but which were considerably nearer to what would be expected from a monochlorcarbinol chloride. A sample of carbinol (m. p. 78°) was analyzed for total halogen by the sodium carbonate method.[1] The bromine was determined by heating the silver halide in a stream of dry chlorine gas:

Weight of substance taken. Gram.	Weight of silver halide. Gram.	Loss in weight after heating with Cl_2. Gram.	Calculated. Bromine in $BrR'R_2COH$. Per cent.	Calculated. Chlorine in $ClR'R_2COH$. Per cent.	Found. Br. Per cent.	Found. Cl. Per cent.
0.3909	0.1757	0.0198	23.59	12.05	9.1	5.83

This analysis and others of the carbinol and carbinol chloride showed that the product from the Friedel and Crafts reaction was not homogeneous but was a mixture of brom- and chlortriphenylcarbinol chlorides whose separation by crystallization was impossible. A blank experiment with a mixture of pure *p*-brom- and *p*-chlortriphenylcarbinol chlorides confirmed this view. The formation of the two monohalogen-triphenylcarbinol chlorides could only be explained by the action of phosphorus pentachloride on the *p*-brombenzophenone. The dichloride obtained from the *p*-brombenzophenone was saponified with sodium carbonate. The product obtained was crystallized several times from petroleum ether and alcohol. The melting-point varied from 73–76.5°. *p*-Chlorbenzophenone melts at 77–78° and *p*-brombenzophenone at 82°. A blank experiment with a mixture of pure *p*-chlor- and *p*-brombenzophenones showed that separation by crystallization was impossible and that the melting-point did not furnish a criterion of purity. Analyses of different samples of the saponified material were made with the following results:

[1] Classen: "Ausgewählte Methoden der Analyt. Chem.," Vol. II, p. 763.

Weight of substance. Gram.	Weight of silver halide. Gram.	Loss of weight after heating in chlorine. Gram.	Calculated. Bromine in p-$BrC_6H_4COC_6H_5$. Per cent.	Calculated. Chlorine in p-$ClC_6H_4COC_6H_5$. Per cent.	Found. Bromine. Per cent.	Found. Chlorine. Per cent.
I. 0.5583	0.3725	0.0449	30.64	16.39	14.45	8.11
II. 0.3902	0.2666	0.0303	.....	.....	14.11	8.21
III. 0.4255	0.2882	0.0282	.....	.....	11.91	9.77
IV. 0.3404	0.2412	0.0211	.....	.....	11.14	11.04

To see if a complete change to a chlor-compound could be effected, the treatment of the *p*-brombenzophenone with phosphorus pentachloride was repeated, using twice the theoretical quantity of pentachloride necessary for replacement of both the oxygen and bromine. The mixture was heated at 150° for ten and one-half hours. The dichloride was saponified and analyzed (see IV of preceding table).

Evidently an equilibrium is reached and to secure complete conversion of the *p*-brombenzophenone to *p*-chlorbenzophenone, long heating with a large excess of the pentachloride would be required.

The replacement of bromine in the benzene ring due to the action of the phosphorus pentachloride is very exceptional. In this connection, it may be mentioned that it has been found in this laboratory that there is very slight, if any, replacement in *p*-dibrombenzophenone. There are several examples in the literature in which there has been a replacement of halogen in the benzene nucleus but we are not aware of one exactly analogous to this case.

R. Benedikt and M. v. Schmidt,[1] by treatment of tribromresorcinol with chlorine gas, obtained a monochlordibromresorcinol. Tribromphenol in hydrochloric acid suspension gave a mixture of chlorine and bromine derivatives which they were not able to separate. O. Srpek,[2] by action of chlorine on bromtoluene, obtained an impure *p*-brombenzylbromide which seemed to have both chlorine and bromine in the nucleus as well as in the side-chain. Kastle and Beatty[3] found that benzenedichlorsulphamide splits off chlorine in the light and under those conditions could replace bromine and iodine from several compounds, *e. g.*, from dibrombenzene, tribromphenol, etc. A. Eibner,[4] in attempting

[1] Monatsh. 4, 604.
[2] Ibid. 11, 431.
[3] Chem. Centr. 1897, p. 578; Am. Ch. J. 19, 139.
[4] Ber. 36, 1, 1229 (1903).

to prepare addition products of chlorine and brombenzene, found that bromine was replaced, especially in the light. Silberstein[1] tried to make the chloride from the nitrate of the diazonium derivatives of *s*-tribromaniline and always obtained an insoluble perbromide. The solution always contained a mixture of chlor- and brom-derivatives. A. Hantzsch[2] found that diazonium chlorides of ortho- and para-di- and poly-brombenzene derivatives in alcoholic solution suffered replacement of bromine by chlorine. R. Wegscheider[3] found that by heating *s*-tribromaniline with concentrated hydrochloric acid at 200°, *s*-trichloraniline was formed. By treating *s*-trichloraniline with concentrated hydrobromic acid under similar conditions, he formed tribromaniline. In neither case did he secure complete conversion although he used 29 molecules of the hydrobromic acid and 26.6 molecules of the hydrochloric acid. Howitz and Witte,[4] by the action of strong hydrochloric acid on certain quinoline derivatives, obtained a change of chlorine for bromine.

4. *Preparation of m-Bromtriphenylcarbinol Chloride by Grignard's Reaction.*—The saponified benzophenone from the dichloride obtained from *p*-brombenzophenone melted usually at 76–77°. This corresponds closely to that of *m*-brombenzophenone (77°). At first it was thought that possibly a shifting of position had occurred. To ascertain if this were the case, *m*-bromtriphenylcarbinol was prepared by Grignard's reaction from the ethyl ester of *m*-brombenzoic acid. The *m*-brombenzoic acid was made from *m*-aminobenzoic acid by Sandmeyer's reaction and was esterified by passing dry hydrochloric acid gas into its absolute alcoholic solution. The ester was distilled *in vacuo* and then in air at 261.2°. The *m*-bromtriphenylcarbinol from Grignard's reaction was changed to the carbinol chloride with hydrochloric acid gas. The melting-point of the carbinol chloride was 67°.

In conclusion we wish to thank Prof. M. Gomberg, of this laboratory, for his many helpful suggestions during the carrying out of this work.

[1] J. pr. Chem. [2] 27, 114 (1883).
[2] Ber. 30, 2, p. 2334.
[3] Monatsh. 18, 329 (1897).
[4] Ber. 38, 1260 (1905).

ANN ARBOR, MICH., February, 1906.

[CONTRIBUTIONS FROM THE HAVEMEYER CHEMICAL LABORATORY, NEW YORK UNIVERSITY.]

ON THE PRINCIPLE OF OPTICAL SUPERPOSITION.

BY M. A. ROSANOFF.

Received January 11, 1906.

(PRELIMINARY COMMUNICATION.)[1]

IN THE celebrated memoir founding the science of stereochemistry,[2] van't Hoff makes an important assumption concerning the optical activities of several asymmetric carbon atoms in a molecule. Referring to molecules made up of two similar asymmetric groups,

$$\begin{matrix} C(R_1R_2R_3) \\ | \\ C(R_1R_2R_3), \end{matrix}$$

he says: "The activity that each of these asymmetric carbon atoms contributes to the whole will be equal or opposite, say a and $-a$; so that the activity of the four isomers will be represented by the expressions: (1) $+a+a$, (2) $+a-a$, (3) $-a+a$, (4) $-a-a$." The assumption symbolized by these expressions is, that the two partial rotations are not themselves changed by addition to, or subtraction from, one another. Writing twenty years later,[3] van't Hoff extends this assumption to molecules with several different asymmetric carbon atoms. "Thus," he says, "for the four pentose types, $COH(CHOH)_3CH_2OH$, we should have the following rotations:

No. 1.	No. 2.	No. 3.	No. 4.
+A	+A	+A	—A
+B	+B	—B	+B
+C	—C	+C	+C."

Here again each partial rotation, A, B, C, is assumed to remain the same, whether the other partial rotations are positive or negative. In other words, *the rotation due to a given asymmetric carbon atom is assumed to be independent of the configuration of the groups around the other asymmetric carbon atoms in the molecule.*

[1] Presented before the New York Section of the American Chemical Society on January 5, 1906.

[2] Bull. soc. chim. [2] **23**, 298 (1875).

[3] Van't Hoff: "Die Lagerung der Atome im Raume," 2d ed., p. 120 (1894); English trans. by Eiloart, p. 160 (London and New York, 1898).

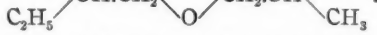
Van't Hoff's assumption was arbitrary. *A priori* it seems possible that a given dextro-rotation, A, might itself be diminished through association with a second dextro-rotation and increased through association with a levo-rotation, or *vice versa*.

In view of the fundamental importance of the assumption, Guye and Gautier[1] decided, in 1893, to subject it to careful experimental inquiry, and during the succeeding three years[2] apparently demonstrated that calculation based on that assumption yields practically the same results as direct observation. Independently of these investigators, and almost simultaneously with them, Walden[3] attacked the same problem, and by an extensive and painstaking series of observations seemed to produce even more brilliant testimony than theirs, in favor of van't Hoff's idea. The assumption, raised to the rank of "The Principle of Optical Superposition," as Guye and Gautier had designated it, was thus incorporated in stereochemistry, and to-day accounts of it may be found in all text-books.

Careful study has now led me to the conviction that a serious error of principle was involved in the experimental work of Guye and Gautier, as well as of Walden, in consequence of which their results have really no bearing on the principle of superposition. On the other hand, the few known facts that do belong within the scope of the principle, not only fail to corroborate it but rather seem distinctly to contradict it.

§1. CRITIQUE OF THE ACCEPTED EXPERIMENTAL MATERIAL.

One of the first cases studied by Guye and Gautier was that of the oxide of two active *l*-amyl radicles (di-*l*-amyl ether):



The two halves of this molecule being similar (as in ordinary tartaric acid), the total optical rotation should, according to the principle of superposition, be, say, $-a-a=-2a$, and each partial rotation should be independent of the other. If, therefore, one of the partial rotations were annulled, the remaining

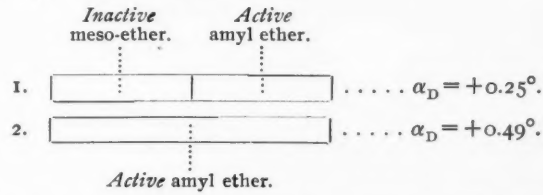
[1] Bull. soc. chim. [3] 9, 403 (1893).

[2] Compt. rend. 119, 740 and 953 (1894); Bull. soc. chim. [3] 11, 1170 (1894); Ibid. 13, 457 (1895). See also Guye and Jordan: Compt. rend. 120, 632 (1895); Guye: Ibid. 121, 827 (1895); Ibid. 122, 932 (1896).

[3] Z. physik. Chem. 15, 638 (1894); Ibid. 17, 720 (1895).

rotation should be exactly one-half the original total rotation. Now, a rotation, $-a$, of *l*-amyl can be counterbalanced by an equal and opposite rotation, $+a$, of *d*-amyl. "Experimentally," the authors say, "this result is obtained by causing active amyl (*i. e.*, *l*-amyl) bromide to act on racemic sodium amylate. Although one isolates, under these conditions, a mixture of the active oxide and the inactive indivisible oxide, this mixture must act in the polariscope as if the optical effect of one of the two asymmetric carbon atoms had been annulled. We have found, for an ether so prepared: $\alpha_D = +0.25°$ in a 0.5 dm. tube....., so that, according to the principle of algebraic superposition, amyl oxide with two identical active carbon atoms must give a rotation.....$\alpha_D = +0.50$.....in a 0.5 dm. tube. This point established, we prepared this latter amyl oxide by the action of active amyl bromide on active sodium amylate (both from *l*-amyl alcohol), and, as a matter of fact, this substance, properly purified, gave a rotation $\alpha_D = +0.49°$ in a 0.5 dm. tube."

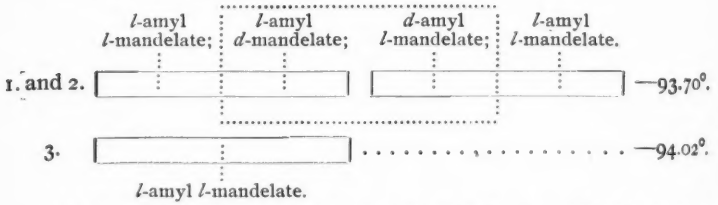
Curiously enough, this result is generally considered as one of the experimental props of the principle of optical superposition. Yet, remembering that one of the two 0.5 dm. tubes contained a mixture, and imagining the ingredients of the mixture as occupying separate halves of that tube, the two polarimetric experiments may be pictured as follows:



Plainly, the rotation in the first tube was one-half that in the second, simply because the first tube contained one-half as much active ether as the second tube. The rotation would still have been one-half if the principle of optical superposition were quite wrong. Assuming the absence of impurities, the slight disparity of the results is due to the fact that in the first tube the active ether was *dissolved* in an equal quantity of inactive ether, the latter having otherwise no more power to counterbalance optical rotation than water or any other inactive solvent.

The rest of Guye and Gautier's observations, and all of Walden's, were made on esters from active alcohols and acids. The theory was that the rotation of an ester with its acid group optically annulled, *plus* the rotation of the ester with its alcohol group optically annulled, must equal the rotation of the ester with neither of its partial rotations annulled, if the principle of optical superposition is correct. Three substances were examined in each case: (1) the ester from an active alcohol and the racemic modification of the acid; (2) the ester from an active acid and the racemic modification of the alcohol; (3) the ester from an active alcohol and an active acid. Thus, to mention one of the many cases investigated by Walden, *l*-amyl *r*-mandelate[1] gave $[\alpha]_D = +2.76°$; *r*-amyl *l*-mandelate gave $[\alpha]_D = -96.46°$. The algebraic sum of the two is $-93.70°$; and in fact, *l*-amyl *l*-mandelate gave $[\alpha]_D = -94.02°$, the slight difference being attributed to unavoidable impurities.

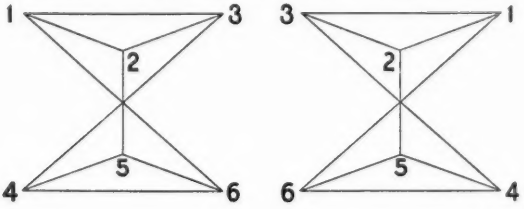
Now, again imagining the ingredients of the mixtures involved as occupying separate halves of the tubes, the three experiments of this case may be pictured as follows:



Adding the first two results algebraically is equivalent to making a joint observation, as pictured. Since *l*-amyl *d*-mandelate and *d*-amyl *l*-mandelate have equal and opposite rotations,[2]

[1] *r* denotes the racemic modification.

[2] That they do have equal and opposite total rotations, whether the partial rotations within each molecule modify one another or not, is obvious from the fact that their molecules are related as an object and its reflected image:



the part of the system enclosed by the dotted lines is optically inactive. But then it is clear that the rotation produced by the first two tubes together equals that produced by the third tube, again not by virtue of the law of optical superposition, but simply because the first two tubes contain together as much active substance (*viz.*, *l*-amyl *l*-mandelate) as the third tube. The slight difference must be due, again, to the solvent influences in the first two tubes.

The cases analyzed above are typical of all the experimental work done in direct connection with the principle of superposition. One further case has been theoretically connected with the principle by Landolt and requires consideration, since different in character from the above. Landolt[1] sees optical superposition in the fact that the molecular rotation of the amyl ester of amylacetic acid equals the molecular rotation of amyl acetate *plus* the molecular rotation of amylacetic acid:

	$[M]_D$.
I. $CH_3COO.C_5H_{11}$.....	+ 3.25°
II. $C_5H_{11}.CH_2COOH$.....	+11.08
I+II.....	+14.33°
III. $C_5H_{11}.CH_2COO.C_5H_{11}$.....	+14.02

The figures are Walden's. But, on the one hand, using another set of Walden's experimental figures, I find no such agreement in the closely analogous case of the amyl ester of diamylacetic acid:

	$[M]_D$.
I. $CH_3COO.C_5H_{11}$.....	+ 3.25°
II. $(C_5H_{11})_2.CHCOOH$	+36.54
I+II.....	+39 79°
III. $(C_5H_{11})_2.CHCOO.C_5H_{11}$...	+37.69

On the other hand, cases like Landolt's, if general instead of exceptional, would lead, not to the principle of optical superposition, but to the theorem that the rotatory power of an active radicle is independent of the chemical composition of the rest of the molecule.[2] Indeed, let the rotations of the amyl radicle, when combined with the several groups involved, be respectively as follows:

[1] "Das optische Drehungsvermögen," 2d ed., p. 267 (Braunschweig, 1898).

[2] Patterson and Taylor (Trans. London Chem. Soc. 87, 33 (1905), seem to think that this is really what is meant by optical superposition.

Amyl with CH_3COO-..... a
Amyl with $-CH_2COOH$..... b
Amyl with $C_5H_{11}CH_2COO-$..... c
Amyl with $-CH_2COOC_5H_{11}$ ..... d

In the case selected by Landolt it happens that $a+b=c+d$, from which he concludes that $a=c$ and $b=d$, *i. e.*, that amyl has the same optical value whether combined with $C_2H_3O_2$ or with $C_7H_{13}O_2$. The error is self-evident.

The accidental equality is readily explained. The reason $a+b$ equals $c+d$ is not that $a=c$ and $b=d$, but that $a<c$ and $b>d$ and the two inequalities nearly balance. Indeed, that the rotation of the amyl group forming esters with fatty acid radicles increases with the mass of the radicles, and is therefore *less* with CH_3COO- than with $C_5H_{11}CH_2COO-$, is shown by the following figures:

Amyl acetate ..... $[M]_D = 3.25°$
Amyl propionate..... 3.99
Amyl butyrate..... 4.25

This points clearly to the inequality $a<c$. On the other hand, the rotation of the amyl radicle of amylacetic acid is known to be *greater* in the acid itself than in its esters; thus,

Amylacetic acid ..... $[M]_D = 11.08°$
Ethyl amylacetate 10.36,

which points to the inequality $b>d$.

§2. ON VAN'T HOFF'S ASSUMPTION.

It is now clear that all the experimental material hitherto brought forward in support of the principle of superposition really fails to throw any light upon it. And so the question is reopened, is van't Hoff's assumption correct or not?

Before searching for an answer, let us formulate the question itself in lucid terms. An asymmetric carbon atom is a carbon atom linked to four different groups. The numerical value of its rotatory power is well known to depend upon the composition and constitution of each of its four groups. Therefore, the question can only be, is the value of the rotatory power independent of the *stereochemical configuration* of the atoms within each of the four groups?

A negative answer is immediately suggested by the well known fact that the configuration of the maleïc and similar acid radicles has a pronounced influence on the rotatory power of *l*-amyl.

Indeed, when in a given case the maleïnoid configuration is changed to the fumaroid (composition and constitution remaining the same), the rotatory power suffers very considerable change.[1]

	$[M]_D$.		$[M]_D$.
l-amyl maleate.....	11.82°	*l*-amyl fumarate.....	15.17°
l-amyl chloromaleate.....	11.70°	*l*-amyl chlorofumarate..	16.78°
l-amyl bromomaleate.....	15.36°	*l*-amyl bromofumarate..	20.07°
l-amyl methylmaleate...	11.17°	*l*-amyl methylfumarate.	16.01°

In cases like these it may be thought that configuration influences the optical effect because the motion ("free rotation") of the radicles is interfered with by the double bond. And so it may still be asked, is optical effect independent of configuration in the absence of double bonds? The *hypothesis* of optical superposition, as already stated, is nothing but an affirmative answer to this question. But it will presently be seen that *facts* furnish a negative answer; in other words, that facts lead to a proposition which is exactly the reverse of the "principle" of superposition.

The facts in question are the rotatory powers of the *l*-amyl esters of dextro, levo- and meso-tartaric acids, which represent the simplest possible, and hence the most reliable case. Let the principle of superposition be true and the amyl rotation independent of the configurations of the acid radicles, and therefore the same in the three, say a. Similarly, let the independent rotation of the dextro-acid radicle be $+b$, that of the levo-acid radicle $-b$, and that of the meso-radicle *zero*. Then the rotations of the three esters should be:

I. *l*-amyl *d*-tartrate..... ..... $a + b$
II. *l*-amyl mesotartrate..... $a + o$
III. *l*-amyl *l*-tartrate..... ..... $a - b$,

and the difference between I and II should be the same as that between II and III.

Now III, the rotation of *l*-amyl *l*-tartrate, has never been actually observed. It can, however, be ascertained by a simple and highly reliable calculation; and that, strange to say, from observations made by Walden himself in connection with that erroneous demonstration of the principle of superposition. Indeed, while the experiments of Guye and of Walden fail to test this principle, they do prove that the rotation of, say, a l_1d_2 com-

[1] Landolt, p. 258. The figures are from Walden: Z. physik. Chem. **20**, 377 (1896).

pound may be very closely calculated from the rotations of l_1r_2 and r_1d_2. Theoretically the question might have arisen as to whether the solvent influences involved would not render the algebraic sum of the rotations of l_1r_2 and r_1d_2 considerably different from the rotation of l_1d_2. But Guye's and Walden's numerous experiments prove conclusively that the solvent influences are slight and that algebraic summation yields highly reliable results. Moreover, since the compound r_1d_2 is the mirror-image of r_1l_2, the rotation of the latter may be obtained by reversing the sign of the rotation of r_1d_2. But then, knowing the rotations of l_1r_2 and r_1l_2, we find, by algebraic summation, a value for the rotation of l_1l_2, which is again very close to the truth. Finally, by reversing the signs of the rotations of l_1d_2 and l_1l_2, we obtain the rotations of d_1l_2 and d_1d_2. *In this manner it is easy to calculate quite exactly the rotations of all the combinations possible in a given case if only the rotations of any two of the combinations of active with racemic radicles are known.*

Walden observed:

r-amyl *d*-tartrate.....	$[M]_D = +40.89°$
l-amyl racemate.....	$+ 9.77°$

Reversing the sign of the *d*-tartrate, we have:

r-amyl *l*-tartrate.....	$[M]_D = -40.89°$
l-amyl racemate.....	$+ 9.77°$

Two algebraic summations now give:

l-amyl *d*-tartrate.....	$[M]_D = +50.66°$
l-amyl *l*-tartrate.....	$-31.12°$

For *l*-amyl mesotartrate Walden observed $[M]_D = +13.83°$. And so we have:

I. *l*-amyl *d*-tartrate.....	$[M]_D = +50.66°$
II. *l*-amyl mesotartrate..	$+13.83°$
III. *l*-amyl *l*-tartrate	$-31.12°$

The difference between I and II is **36.83**; the difference between II and III is **44.95**. The considerable disagreement points clearly against van't Hoff's assumption and in favor of a general principle that may be provisionally stated as follows:

The optical rotatory power of an asymmetric carbon atom depends upon the composition, constitution, AND CONFIGURATION *of each of its four groups.*

The same conclusion is reached, in a slightly different way, by comparing the rotations of *l*-amyl mesotartrate and *l*-amyl

racemate. Assuming the rotation of the racemate to be very nearly one-half the sum of the rotations of *l*-amyl *d*-tartrate and *l*-amyl *l*-tartrate,[1] the rotations of the racemate and the meso-tartrate should be *very nearly* the same if the principle of superposition were correct. In reality Walden found:[2]

l-amyl racemate.....	$[M]_D = 9.77°$
l-amyl mesotartrate.....	$13.83°$

The difference is great.

Less decisive is the case of the *l*-amyl esters of the racemic and meso-modifications of dimethylsuccinic acid.[3] Here the molecular rotations found by Walden are, respectively, 10.47° and 9.79°. The difference, however, which happens to be small, might be due either to the influence of configuration or to extra-molecular influences in the racemate mixture. The point can only be decided by examining the esters of the *d*- and *l*-acids separately.

I am instituting a series of experiments, designed to further test the principle provisionally formulated in this paper, and hope to communicate the results before very long.

It is a pleasure, in concluding, to acknowledge my indebtedness to Professor Morris Loeb, of this University, for a number of important suggestions concerning both the subject-matter of the present paper and its presentation.

DETERMINATION OF THE SODIUM PHOSPHATES.

By C. Chester Ahlum.

Received January 10, 1906.

Because of the close similarity of the reactions of disodium hydrogen phosphate and trisodium phosphate, the quantitative determination of these salts when in mixture requires special and modified methods.

It is known that both of these phosphates may be titrated with standard acid and if isolated may be determined with accuracy by this means. Both disodium hydrogen phosphate and tri-

[1] This assumption, again, is justified by the very observations that were intended to prove the principle of superposition. It is further justified by the observations of Hammerschmidt (see Landolt: loc. cit. p. 213).

[2] Loc. cit.

[3] Landolt: loc. cit. p. 258.

sodium phosphate are alkaline to methyl orange while sodium dihydrogen phosphate is neutral; therefore, the point of neutralization or end reaction occurs when either or both of the alkaline phosphates are converted into the acid phosphate.

$$HCl + Na_2HPO_4 = NaH_2PO_4 + NaCl$$
$$2HCl + Na_3PO_4 = NaH_2PO_4 + 2NaCl.$$

From the above reactions it is observed that trisodium phosphate requires two molecules of acid while the disodium hydrogen phosphate requires but one. Therefore, 1 cc. of N/10 hydrochloric acid will be equivalent to 0.0082 gram of Na_3PO_4 or 0.0142 gram of Na_2HPO_4.

If carbon dioxide is passed into a solution of trisodium phosphate the latter will be converted into disodium hydrogen phosphate with the formation of a definite quantity of sodium carbonate,

$$CO_2 + 2Na_3PO_4 + H_2O = 2Na_2HPO_4 + Na_2CO_3.$$

As the sodium carbonate formed is directly proportional to the trisodium phosphate, a determination of the latter salt, when in mixture with disodium hydrogen phosphate, can be very easily accomplished, *i. e.*, by passing carbon dioxide into a solution of the mixed salts and determining the sodium carbonate formed.

Two grams of the mixed salt are dissolved in water and carbon dioxide passed through the solution until the reaction is complete (about ten or fifteen minutes). The solution is then evaporated to dryness and the sodium carbonate estimated by the Schrötter apparatus. The amount of carbon dioxide eliminated multiplied by 7.4545 gives the amount of trisodium phosphate present in the mixture.

One gram of the mixed salts is dissolved in water and titrated with N/10 hydrochloric acid. The number of cubic centimeters required by the trisodium phosphate is obtained by dividing the amount (in grams) of trisodium phosphate found in the above determination by 0.0164 (2×0.0082). The number of cubic centimeters required by the disodium hydrogen phosphate is the difference between the number obtained in the titration and the number required by the trisodium phosphate. The number of cubic centimeters required by the disodium hydrogen phosphate multiplied by 0.0142 gives the amount of disodium hydrogen phosphate in the mixture.

If the original mixture contains sodium carbonate, a determination of this ingredient will be necessary in order to estimate the

amount of sodium carbonate formed from the trisodium phosphate and also to make a correction in the titration.

Alternative Method.—If a solution containing disodium hydrogen phosphate and trisodium phosphate is acidified and then neutralized with sodium carbonate, adding an excess of the latter, we have as a result a solution containing disodium hydrogen phosphate and sodium carbonate.

If a titration were to be made of the mixed salts before treatment and a titration made after, making allowance for the amount of sodium carbonate found in excess, we find the amount of acid required by the phosphates after treatment is less than that required before, the difference being directly proportional to the amount of trisodium phosphate present.

This is made clear by referring to the reactions above, noting that disodium hydrogen phosphate requires but one molecule of acid while the trisodium phosphate requires two.

This method in detail is as follows: 0.5 gram is dissolved in 50 cc. of water and titrated with N/10 hydrochloric acid. 1.0 gram is dissolved in 50 cc. of water containing a drop of methyl orange. Hydrochloric acid is added in slight excess and the solution boiled for ten minutes. Sodium carbonate is added in excess and the solution concentrated by boiling as far as possible. It is then transferred to a weighed platinum dish, evaporated to dryness on a steam-bath, dried in an oven and weighed.

The mass in the dish is broken up and pulverized with a porcelain pestle, guarding against loss. One-half of the amount of solid matter found is weighed off and the carbon dioxide determined by the Schrötter apparatus. The remaining half of the solids is dissolved in 50 cc. of water and titrated with N/10 hydrochloric acid.

The following example will be explanatory: 0.5 gram was titrated with N/10 hydrochloric acid, requiring 23.1 cc. One gram was treated in the manner described above, titrating a solution of one-half of the solids and estimating the carbon dioxide in the other half. The amount of carbon dioxide found was 0.0149 gram. The amount of sodium carbonate equivalent to the carbon dioxide eliminated $=0.0149\times 2.4115=0.0359$ gram.

The number of cubic centimeters of N/10 hydrochloric acid equivalent to the sodium carbonate present = 0.0359 ÷ 0.0053 = 6.7.

The number of cubic centimeters of N/10 hydrochloric acid required after treatment was found to be 20.1.

	cc.
Total N/10 acid required	20.1
N/10 acid required by Na_2CO_3	6.7
N/10 acid required by Na_2HPO_4	13.4

	cc.
N/10 HCl required by the $Na_3PO_4 + Na_2HPO_4$	23.1
N/10 HCl required by the Na_2HPO_4 (the original Na_2HPO_4 with that formed from the trisodium phosphate)	13.4
Difference	9.7

The difference in the amount of acid required is due to the loss in alkalinity of the trisodium phosphate, caused by the conversion of this salt into disodium hydrogen phosphate, thereby requiring just one-half of the acid as when in the tribasic state.

In the above example the amount of N/10 hydrochloric acid necessary to completely act upon the trisodium phosphate would be $9.7 \times 2 = 19.4$ cc. As each cubic centimeter is equivalent to 0.0082 gram of trisodium phosphate, the amount of this salt in the mixture will be $19.4 \times 0.0082 \times 200 = 31.9$ per cent.

The number of cubic centimeters required by the disodium hydrogen phosphate is obtained by subtracting the number of cubic centimeters required by the trisodium phosphate from the number of cubic centimeters obtained in the original titration.

In the above example this would be 23.1 cc.—19.4 cc. = 3.7 cc. As each cubic centimeter of N/10 hydrochloric acid is equivalent to 0.0142 gram of disodium hydrogen phosphate, the amount of this salt present in the mixture would be $3.7 \times 0.0142 \times 200 = 10.5$ per cent.

The accuracy of the determinations depends upon the determination of the sodium carbonate. As all sources of error may be traced to this determination, this should be performed with care and results checked.

A number of mixtures made up of disodium hydrogen phosphate and trisodium phosphate of known purity, were analyzed

according to this method, with results tabulated below. Both salts employed were chemically pure crystals containing twelve molecules of water.

Per cent. known to be present.		Per cent. found.	
Na_3PO_4.	Na_2HPO_4.	Na_3PO_4.	Na_2HPO_4.
8.63	31.72	8.11	32.81
12.94	27.76	11.23	28.46
17.26	23.79	17.98	23.10
23.74	17.84	24.21	16.89
30.11	11.89	31.93	11.09
32.37	9.92	32.71	10.16
34.52	7.93	33.32	9.11
36.68	5.94	36.84	6.17

If alkaline carbonates are present in the original salts they must be determined by the Schrötter apparatus and the necessary correction applied to the titration.

LABORATORIES OF GEO. W. LORD COMPANY, PHILADELPHIA.

A DESCRIPTION OF IMPROVED APPARATUS AND OF A MODIFICATION OF DREHSCHMIDT'S METHOD FOR THE DETERMINATION OF TOTAL SULPHUR IN COAL GAS.[1]

BY EVERHART PERCY HARDING.

Received January 11, 1906.

OF ALL methods[2] discussed and used during the last half century for the determination of total sulphur in coal gas the one proposed and used by Drehschmidt appears[3] to be the most convenient and to give the most reliable results.

The method of Drehschmidt consists in the oxidation of the sulphur to sulphur dioxide in a supply of air freed from hydrogen sulphide, of the conversion of the sulphur dioxide into potassium sulphite by its absorption in a 5 per cent. solution of potassium carbonate, of the oxidation of the sulphite to potassium sulphate with bromine and of the subsequent precipitation of the sulphur with a solution of barium chloride, and the calculation of the amount of sulphur from the weight of the ignited barium sulphate.

[1] The writer is indebted to Mr. O. L. Bernhagen for the drawing of the apparatus used in this paper.

[2] Chem. News, 1861, p. 38; 1863, p. 73; 1868, p. 89; Z. anal. Chem. **15**, 175; **21**, 335; **22**, 171. Hempel: "Gas Analyse," Dritte Auflage, s. 304.

[3] Chem. Ztg. **11**, 1382 (1887.)

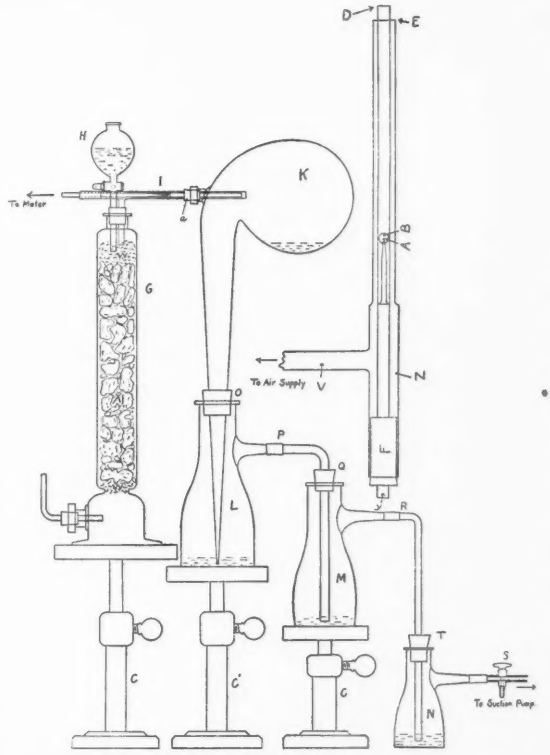
The apparatus used by Drehschmidt[1] consists of a gas meter for measuring the gas, of a drying tower containing pieces of pumice stone saturated with a solution of potassium hydroxide for removing hydrogen sulphide from the air, of a specially constructed Bunsen burner for burning the gas, of a cylindrical glass mantle which rests in a mercury seal and which encloses the burning gas and conducts the products of combustion to the absorption apparatus, of an absorption apparatus which is connected at one end by a ground glass joint to the upper end of the mantle which terminates in a glass tube bent downward and at the other end to an aspirator, and of an aspirator to produce a draft and to draw the products of combustion through the absorbing solution.

The writer of this paper has always experienced difficulties in manipulating Drehschmidt's apparatus. In the first place it was always difficult to regulate the gas supply and to control the aspiration so as to insure both a complete consumption and a continuous combustion of the gas. In the second place, the moisture of combustion produced during the first part of the determination would condense on the walls of the mantle holding some sulphur dioxide in solution, collect into large drops and flow down into the mercury seal, thus insuring loss of sulphur dioxide. Other objections to this apparatus are its delicately constructed absorption flasks and the non-flexible ground glass joint connecting the mantle with the absorption apparatus which, in the hands of inexperienced students, may be easily broken and which can be replaced only at an expense. Lastly, it is expensive.

The principle of the method in use by the writer consists in the combustion of the gas in a specially constructed hard glass burner within the body of a large retort in the presence of bromine vapors, the aspiration of the products of combustion together with some bromine vapors through a 5 per cent. solution of potassium carbonate, and the precipitation and weighing of the sulphur as barium sulphate. The apparatus with the exception of the burner is of the simplest construction and can be set up at once in any working laboratory.

A description of the apparatus is as follows: G is a purifying

tower 45 cm. high and 6.3 cm. in diameter filled with pumice stone contained between the glass wool layers WW. H is a dropping funnel for delivering continuously the potassium hydroxide solution. K is a retort 24 cm. in depth, 16 cm. in cross-section with a delivery tube 46 cm. in length and which is fitted into the



IMPROVED APPARATUS FOR THE DETERMINATION OF TOTAL SULPHUR IN COAL GAS

flask L by the rubber stopper O. L, M and N are heavy glass suction bottles with the respective diameters 10 cm., 8.9 cm., 6.3 cm., and are connected at P and R with closely fitting rubber tubing. C, C′ and C″ are adjustable wooden supports. S is a glass cock connecting the apparatus with the suction pump for

minutely adjusting the aspiration. I is a burner connected at *y* with the meter at V by means of a short piece of rubber tubing, with the purifying tower G, and which is fitted into the tubule of the retort with the rubber stopper *a*. The burner[1] which is represented in the drawing is made of hard glass tubing. E the air supply tube is 9 mm. in diameter at E and 12 mm. at Z. D the gas supply tube is 5.8 mm. in diameter with opposite holes B for the admission of air and drawn out at A to an opening sufficiently small to deliver, under aspiration, between 0.35 and 0.5 cubic foot of gas per hour.

To make a total sulphur determination the pumice stone is saturated with a 33⅓ per cent. solution of potassium hydroxide and the solution allowed to drop slowly and continuously from G during the process. The burner is connected with the meter and the tower, the gas turned on, ignited and allowed to burn for fifteen minutes. The retort and the absorption flasks in the meantime are cleaned and supplied with a 5 per cent. solution of potassium carbonate, 30 cc. being put into N, 30 cc. into M, 50 cc. into L, and 30 cc. together with 4 cc. of bromine into the retort, K, and the flasks and retort connected as represented in the figure. The gas is then turned off, the burner inserted into the tubule of the retort and air aspirated for ten minutes through the apparatus. The aspiration is then momentarily discontinued, the burner removed from the retort, the gas turned on and ignited, the burner reinserted and the aspiration continued and regulated with the cock S.

After 1½ to 2 cubic feet of gas have been burned at the rate of 0.35 to 0.5 cubic foot per hour the gas is turned off and the aspiration continued until the apparatus cools down to room temperature. The burner is then withdrawn from the retort and rinsed with distilled water into a 500 cc. beaker. The retort and absorption flasks are disconnected, their contents poured into the beaker and then rinsed several times with small portions of distilled water. The solution is then acidified with hydrochloric acid, concentrated to a bulk of 100 cc., then transferred to a No. 3 beaker and the sulphur precipitated and weighed as barium sulphate by the usual method.

[1] This burner and others used with this apparatus were made by Francis C. Frary, Assistant in Chemistry at this University.

This apparatus has advantages over Drehschmidt's apparatus. It is inexpensive. The absorption apparatus and the retort combustion chamber are inexpensive and common apparatus of every laboratory. There are no non-flexible ground glass joints and no sulphur dioxide is lost by the condensation of moisture during the first part of the determination. The only joints the combustion gases come in contact with are the ones connecting the absorption flasks and in these the glass tubes are in close contact with each other. The burner is so small in diameter and has such a small opening at A and the gas is so well mixed with air at B that a complete consumption and a continuous combustion of the gas are assured. The flame withstands a strong aspiration and the opening A is so small as not to allow an excess of gas. The relative positions of the gas tube and air supply tube are adjustable so that if the openings A were too large for the opening B, D could be brought nearer to E thus insuring a greater supply of air for the combustion, but the flame would then be more easily extinguished and would not withstand such a strong aspiration. The aspiration is regulated to a nicety with the cock S.

The advantage of this method over that of Drehschmidt is the combustion of the gas in the presence of bromine vapors which immediately oxidize the sulphur dioxide in the presence of the moisture of combustion to sulphuric acid which is then converted into potassium sulphate, thus insuring no loss by an incomplete absorption of the sulphur dioxide. The heat of combustion vaporizes the bromine sufficiently rapidly to maintain a constant vapor in the retort. These vapors are sparingly carried through the apparatus so that no sulphur dioxide can possibly escape oxidation.

Hempel[1] has modified Drehschmidt's apparatus by burning the gas in a glass burner within a receiver flask but he uses the same expensive and delicately constructed absorption apparatus.

[1] Hempel: "Gas Analyze," Dritte Auflage, s. 304.

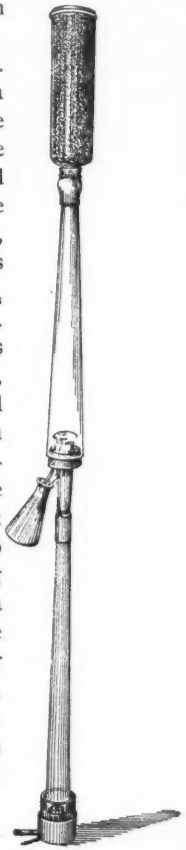
UNIVERSITY OF MINNESOTA.

DETERMINATION OF TOTAL SULPHUR IN ILLUMINATING GAS.

By Charles D. Jenkins.

Received December 20, 1905.

The apparatus here described has been in use in Massachusetts for some years for obtaining the amount of sulphur, in all forms, in illuminating gas. A feature of the apparatus, which in its original form was devised by C. W. Hinman and since improved by the writer, is portability; in its present form only three or four minutes are required to set up or take down the apparatus ready for traveling.



The upper vessel is a "bead glass" 300 mm. long and 60 mm. in diameter; this is filled with large cut glass beads, held up by a suitable fluted glass, giving a large condensing surface without obstructing the draft. To this bead glass is attached, by a rubber connector, the upper adapter, 410 mm. long and 50 mm. lower, internal diameter. To the upper adapter is attached, by means of the "connecting piece," the lower adapter, 400 mm. long and 40 mm. lower diameter. The connecting piece projects 12 mm. above the top of a rubber stopper, fitting the upper adapter, and is surmounted by a watch-glass deflector, carried on platinum wires. An overflow tube carries the condensation to the Erlenmeyer flask hung on the stopper as shown; this tube is so adjusted that some liquid remains on the stopper, to keep it cool and to absorb some of the ascending gases. The Bunsen burner is fitted with a lava tip having a 5 mm. hole; surrounding the burner is a glass tube 20 mm. in diameter forming the inner wall of an annular chamber, of which the outer wall is a glass ring 50 mm. in diameter. Into this chamber, which serves to contain 10 per cent. ammonium hydroxide, the lower adapter dips 10 mm.

The gas to be tested is measured through a

small dry gas meter and then burned at the bottom of the system of condensers at the rate of 0.6 cubic feet per hour. All the air supplied to the burner mixing tube and to the flame must pass over the ammonia in the annular chamber, thus insuring an ammoniacal atmosphere throughout the apparatus. Ten per cent. ammonium hydroxide is added at the rate of 2–3 cc. for each 0.2 cubic foot of gas burned. The various sulphur compounds burn to sulphur dioxide and sulphur trioxide, which, combined with the ammonia, are dissolved in the condensed water and collected; the apparatus is washed out with 200 cc. of distilled water.

The sulphur is determined as sulphuric acid by a volumetric chromic acid method worked out by Hinman[1] in 1877. Andrews[2] and Pennock[3] have described a similar method. To the solution and washings obtained from burning 0.8 to 1.0 cubic foot of gas are added 2-3 cc. bromine water and the solution evaporated to 30–40 cc. in bulk. To this hot solution is added an excess of a hydrochloric acid solution of barium chromate; it is gently boiled, an excess of dilute ammonia added, boiled again for a minute, filtered and washed. The ammonium chromate in the filtrate (the chromic acid being equivalent to the sulphuric acid in the original solution) after being boiled in a stout flask with a Bunsen valve, to expel air, is cooled and titrated direct with stannous chloride, using the iodo-starch blue to accentuate the end-point.

A weak sulphuric acid was analyzed by different methods with the following results:

	Gram.
The usual gravimetric method gave..... ...	0.04772
Titrating against sodium carbonate.....	0.04783
The above volumetric method gave.....	0.04804

Four sets of apparatus were run in parallel with a common gas supply. The sulphur being determined as above, gave grains per 100 cubic foot of gas: 12.26, 12.29, 12.23, 12.26.

Ten cubic feet of gas were burned in several apparatus and the sulphur determined, in duplicate, gravimetrically and volumetrically, as follows:

	Grains per 100 cu. ft.	Grains per 100 cu. ft.
Gravimetrically.....	10.97	10.97
Volumetrically.....	11.01	11.04

[1] Amer. J. Science and Arts, 14, 478.
[2] Am. Ch. J. 11, 567; 32, 476.
[3] This Journal, 25, 1265.

With this apparatus and method of analysis sulphur in illuminating gas can be rapidly and accurately determined in a small sample of gas.

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A SYSTEM OF RECORDS FOR ANALYTICAL LABORATORIES.

BY R. W. THATCHER.

Received December 26, 1905.

A SATISFACTORY system of records for an analytical laboratory requires that all the data concerning each sample which is analyzed shall be in one place, that this information may be easily found when needed, and that the records shall be in some compact form which can be conveniently preserved in some indestructible and fire-proof place. A further convenience is attained if the record of the sample and the results of its analysis appear together in some complete and concise form. A great saving of time and labor is insured if the records are so arranged that the terms used in recording and reporting results of analyses are in printed form, thus requiring only the filling in of the analytical numbers in their proper places. A system which embodies all these advantages was devised by the writer and has been in use in the chemical laboratory of the Washington Agricultural Experiment Station for the past two years, and has given such excellent satisfaction that a brief description of it is offered below with the idea that its use may be of very general application and advantage.

The general plan of these records is a modification of the card index system which is so universally used. A set of cards, five by eight inches in size, was obtained. On one face of each card was printed a blank form for recording the origin of the sample, its description, the date of its receipt, the date of analysis, and the signature of the analyst; and beneath this the statement of the results of the analysis. The set of cards in use in this laboratory is printed in five different forms as shown in the accomcompanying illustration. These forms are appropriately printed for recording analyses of "Soils," "Fertilizers," "Feeding Stuffs," "Foods" and "Miscellaneous" samples. Each form is printed on cards of a different color, a great convenience in

carrying the proper cards with their respective samples in the laboratory. The reverse side of the cards is left blank and is used for recording analytical data.

When a sample is received at the office, its description, and the laboratory number assigned to it are recorded on a card of the proper form. This card is then carried into the laboratory

and all analytical data in the form of weights, burette readings, etc., are recorded on the back of the card, together with all calculations of percentages, etc. A small box fastened to the wall near the balance case and another on the burette table serve to hold the cards when not in use and prevent their becoming soiled or injured by chemicals, etc. When the analysis is completed, the results are entered on the face of the card and the card is then filed away in a suitable filing cabinet. The filing cabinet is provided with a set of guide cards, one of which is inserted before each fifty of the record cards. The guide cards are ruled into fifty spaces, each of which contains a laboratory number and a brief description of the sample to which it is assigned. They thus serve the double purpose of regulating the assignment of laboratory numbers to samples as received and of indexing the fifty record cards which follow. This makes it easy to locate any given card for reference at any future time.

For cards printed in the form shown in the illustration a vertical filing case with vertical guide cards would be slightly more convenient than the form of cabinet in which the cards lie horizontally, although the latter form is now in use in this laboratory without any serious inconvenience. The vertical filing cases are not usually kept in stock by dealers in these supplies, and if made to order are somewhat more expensive.

For analytical laboratories other than those in agricultural chemistry, the cards should, of course, be printed in other forms than those described above. In some agricultural chemical laboratories further blank forms for "Insecticides and Fungicides," "Waters," etc., might be added with advantage. The writer would be pleased to send a sample set of his own cards to any one who is interested in the matter, or who contemplates making use of this system.

LABORATORY OF
WASHINGTON AGRICULTURAL EXPERIMENT STATION,
PULLMAN, WASH.

NEW BOOKS.

PROGRESS IN ALKALOIDAL CHEMISTRY DURING THE YEAR 1904. By H. M. GORDIN. Milwaukee, Wis.: Pharmaceutical Review Pub. Co. 1905. 94 pp. Price, $0.70.

This is Monograph No. 10, of the Pharmaceutical Science Series edited by Edward Kremers.

Many important contributions to the chemistry of the alkaloids were reported in 1904. The constitution of ricinine was definitely established. The researches of Knorr and others resulted in throwing additional light upon the probable structure of morphine and of certain of the other opium alkaloids. Investigations of conhydrine and the coniceines brought the problem of their constitution much nearer to its final solution. The identity of lupinidine and sparteine was shown by Willstätter and his co-workers. Papaverine and cotarnine received considerable attention, and many interesting new derivatives of these bases were prepared. New characteristic color reactions for various alkaloids were reported by Reichard and others. Additional data were secured of the distribution of the various alkaloids in the plant kingdom. The only alkaloid discovered in 1904 was skimmianine, which was isolated by Honda from the leaves

of *Skimmia Japonica* Thunb., and to which the formula $C_{22}H_{29}O_9N_3$ was assigned.

The alkaloids are classified alphabetically. The subject-matter is well presented, with full references to the literature, and the monograph constitutes an excellent resumé of the progress of alkaloidal chemistry in 1904. It should be on file in all laboratories interested in this field of organic chemistry.

MARSTON TAYLOR BOGERT.

EXERCISES IN QUANTITATIVE CHEMISTRY. By HARMON NORTHROP MORSE, Professor of Analytical Chemistry in the Johns Hopkins University. Boston: Ginn and Co. 1905. 556 pp. Price, $2.00.

This book is a highly satisfactory collection of well explained quantitative exercises. It is noteworthy for combining many physico-chemical measurements with the most varied exercises in quantitative analysis. The chapter headings include: The balance, barometer, thermometer; calibration of apparatus for measuring gases and liquids; standard solutions; determination of specific gravities and molecular weights; purification; estimation of silver, the halogens, sulphur, nitrogen, phosphorus, arsenic, silicates, carbon dioxide, carbon, hydrogen, alkalies and alkaline earths; gas analysis; volumetric analysis; electro-analysis; butter analysis; electrical heating appliances; electrical methods for combustion of organic substances.

The author thinks that a list of this sort will give an average student the best possible foundation for subsequent work, whether in physical, organic, inorganic or *analytical* chemistry. But in the opinion of the reviewer the list is much longer than could possibly be assigned even for the bulk of a full year's work. In the next place it merely takes little slices from courses, units in themselves, which the student should have later anyway, *e. g.*, physical or organic chemistry. Viewed in this light, therefore, the course seems adapted for students who might not go further in some of the fields treated, *e. g.*, gas analysis; but candidates for the doctorate would secure a more symmetrical development by taking each special field in turn, after an early course in gravimetric and volumetric analysis.

But this list is a step in the right direction. Here are taught varied principles, together with varied quantitative methods. The scrutiny of instruments and their calibration belongs here, as befitting quantitative work, for instruments often tell as wrong

stories as chemical labels. Only by realizing all of these matters does a student see the meaning of the *tenths of a per cent.* The electrical way of making organic combustions seems worthy of general adoption.

This shows the spirit of the book. With the good points, however, some mistakes ought to be pointed out. There is no distinction between work and energy, so the watt is wrongly defined; it measures the rate of consumption or production of energy, *i. e.*, it is a joule per second. It should be stated that Thomson's rule gives only an approximate decomposition value (p. 494). On p. 24, if the weights of a set have been calibrated with one another as they should be, it is *not* necessary to consider the different densities of the small platinum and large brass weights, to reduce to vacuum, for the difference in buoyancy is automatically eliminated by the calibration. Silver chloride can be better washed by decantation than by rinsing it after it is on the Gooch filter. In electro-analysis no mention is made of the enormous saving of time effected by a rotating anode. A mercurous salt is better than ferric chloride for purifying mercury and of course its mercury is regained as pure substance. Nine significant figures for an oxygen equivalent on p. 456 look bad. Atomic weights should be on the oxygen basis.

Of course this work makes no attempt to supplant a great many special treatises. On the whole, teachers ought to find this a suggestive book, and students could profit much by owning it.

ROGER C. WELLS.

CHEMISCHE-TECHNISCHE UNTERSUCHUNGSMETHODEN, HERAUSGEGEBEN VON DR. GEORG LUNGE. Vol. III, with 119 cuts and 3 plates. 8vo. pp. xxvii+1305+44. Fifth completely revised and enlarged edition. 1905. Berlin: Julius Springer. Price, 28.50 marks.

This work is so universally and favorably known that the reviewer deems it best to show wherein it differs from the preceding edition.

The chapters on Oils, Fats and Waxes and Special Methods in the Oil and Fat Industry by Lewkowitsch*, Manchester, on Rubber and Rubber Goods by Frank and Marckwald of Berlin, and on Beer by Prof. Lintner, Munich, are new, these writers contributing for the first time to the treatise.

Other subjects and authors are as follows: Mineral Oils and Lubricants,* Prof. Holde, Berlin; Drugs, Resins, Balsams and

Gums,* Dr. Dieterich, Helfenberg; Essential Oils,* Dr. Gildemeister, Leipzig; The Sugar Industry,* Prof. E. von Lippmann, Halle; Starch, Prof. von Eckenbrecker, Berlin; Alcohol, Dr. Ebertz, Hohenheim; Brandy, Liquors and Vinegar, Dr. Schüle, Strassburg; Wine, Prof. Windisch, Hohenheim; Tannin, Etc., Dr. Councler, Münden; Leather,* Dr. Paessler, Freiberg; Paper,* Prof. Herzberg, Berlin; Inks,* Director Schluttig, Dresden; Organic Preparations, Dr. Messner with Merck in Darmstadt; Citric and Tartaric Acids, Dr. Rasch, Berlin; Organic Dyestuffs, Textiles and Printing, Prof. Gnehm, Zürich.

Many of these writers, notably those starred, have written monographs upon their respective subjects and are among the highest authorities in their specialties. The volume has been increased by about 20 per cent. over the fourth edition and represents the very latest and best work upon those subjects.

While it may be of advantage in some cases to have books unbound (geheftet) yet the writer feels, particularly in books which are sent for review, that these at least should be bound, so that they may be inspected at once without requiring the trouble either of cutting leaves or binding. A. H. GILL.

UNTERSUCHUNG DER MINERALÖLE UND FETTE SOWIE DER IHNEN VERWANDTEN STOFFE, MIT BESONDERER BERÜCKSICHTIGUNG DER SCHMIERMITTEL. By DR. D. HOLDE, Professor, Director of the Royal Testing Station at Grosse Lichterfelde and Docent at the Technical High School, Berlin. Second edition, 8vo. pp. 408 with 99 figures. 1905. Berlin: Julius Springer.

The present edition represents about double the material contained in the former one, the increase relating not so much to the mineral oils as to the allied subject of lubricants.

The work is divided into four parts: 1, Petroleum; 2, Tars and Pitches; 3, Saponifiable Fats and Waxes; 4, Technical Products from Fats and Waxes. An excellent feature of the book is the fact that all the methods described have been tested under the supervision of the author, some of them being original.

The part relating to petroleum constitutes rather more than half of the volume; the subjects considered are, crude petroleum, its testing and evaluation particularly on a semi-industrial scale with a metal retort rather than a glass boiling flask. A full discussion follows of the tests applied to benzines, kerosenes, gas and lubricating oils; fuel, cleaning, and "water-soluble" oils; vaseline and

pitchy residues also find mention. The methods detailed relate more particularly to the German petroleum industry and requirements.

Part II, of about twenty pages, deals with various tarry products from brown coal, shale, and peat and also with ozokerite.

In Part III the saponifiable waxes and fats are discussed—making up nearly one-fourth of the volume. The discussion embraces the preparation and extraction of these oils and the usual physical and chemical tests.

Part IV treats of the tests to be applied to the technical products obtained from the three preceding; *viz.*, candles, wool oil, soaps and soap powders, Turkey-red oil, varnishes and lacquers, blown oils, dégras, linoleum and rubber substitutes.

A useful feature of the book is the system of tables of the preparation and properties of the various oils and of certain lubricants which have proved their value; also the requirements of various German railroads for their oils.

The work contains much valuable information not to be found elsewhere and may be warmly recommended to all interested in the subjects treated. A. H. GILL.

EXPERIMENTAL ELECTROCHEMISTRY. By N. MONROE HOPKINS, PH.D. New York: D. Van Nostrand & Co. 284 pp. Price, $3.00.

This book was evidently written by one who has experimented upon many of the subjects discussed. Photographic reproductions and drawings are very frequently used. It gives the impression that the author was willing to base it upon present theories of electrolytic dissociation, and unwilling to neglect many of the questions which their use force upon a reader. Probably most readers of the average book on the subject have asked themselves: Can an electric current be shown in an electrolytic conductor without electrodes? Is gaseous conduction similar to electrolytic conduction, etc. Such matters are considered experimentally at some length.

No references to the literature are made in the text, though an extensive and general electrochemical bibliography forms a part of the book. The discussion of electrodeless conduction makes it appear that the author is the originator of the scheme for the production of alternating currents in solutions by magnetic induction. Hering outlined these general processes in the *Trans-*

actions of the American Institute of Electrical Engineers four years ago, nor were they new at that time.

Among the typographical errors to which attention should be called is the expression for Ohm's Law, p. 14, and the incomplete chemical symbol on p. 217.

The full description under each illustration is very useful.

Many of the successful, as well as unsuccessful commercial electrolytic processes are considered in detail, often accompanied by descriptions of the author's experiments along the same line.

Among the "definitions of new terms" we find: "Elevation of the boiling-point, often expressed in works on physical chemistry as 'the lowering of the vapor-tension of the solvent.'"

The statement (p. 32) "Raoult,.....to make a long story short, found that all non-electrolytes of equal concentration lowered the freezing-point of pure water to the same extent" is too short to be true. Also: "Raoult.....found 1.85° C. .....and we may say, therefore, that all..... lower the freezing-point of water 1.86° C." Possibly this is permissible if one does not care what he says within one part in 186. On p. 33 we are apparently told that Raoult gave us the same depression.....as a gram-molecule of an electrolyte, which can hardly be allowed even metaphorically. Dangerous abbreviation is disclosed on p. 38, where we are told that for the heat of neutralization "it matters not what acid or base we use or what salt is formed, we always get experimentally..... 13.700 calories." Hydrofluoric acid and ammonia are too common reagents to be thus slighted.

On p. 53, we learn that ions "then become ordinary atoms of hydrogen and may readily be seen in the capillary"; on p. 55 that "the electricity from a frictional machine is almost all potential difference!"

On p. 86, "The errors in weighing a decided increase in the respective cathodes being less than in weighing a slight increase." "There can be no electrolytic conduction without the corresponding setting-free of substances in the ratios of their chemical equivalents" (p. 86) is a careless statement in the light of his detailed experiments with induced currents where the opposite is proved.

On p. 248, in charging a storage battery "oxygen and hydrogen are of course given off from both electrodes."

On p. 253, "It has been found that a couple made of bismuth and antimony, heated at the point of union, corresponds very closely in electromotive force to a couple consisting of zinc and copper immersed in sulphuric acid." In fact, this thermo-potential cannot be over a few hundredths of that of the zinc-copper-acid combination.

The problem of electricity from carbon is given a short chapter, in which all the energy thus far derived from carbon cells is attributed to thermo-electric operations. The manufactures of ozone and nitric acid from the atmosphere are given quite extensive treatment.

Chapter XVI is devoted to the primary cell. No attempts are made to introduce the usual mathematical formulas expressing relationship between ionic concentrations, migration rates and electromotive forces. The familiar and incorrect system of calculating electromotive force corresponding to a given reaction, from heats of formation alone, is given in this chapter and is also previously used to determine the minimum electromotive force necessary to produce a current through an electrolyte.

W. R. WHITNEY.

A SYNOPSIS OF A COURSE OF LECTURES IN GENERAL CHEMISTRY PUT IN THE FORM OF QUESTIONS. By S. L. BIGELOW. Ann Arbor: George Wahr. 1905. 104 pp. Price, 50 cents.

The author's attempt to furnish a series of questions, that shall help a student assimilate the facts acquired in the lecture room and direct his study of some text-book to the best advantage, is very successful. The questions are well chosen, clearly put, and broad enough in their scope to satisfy the demands of most elementary courses. A few tables of constants, and some hints regarding problems concerning gases and solutions are placed in an appendix.

L. B. HALL.

RECENT PUBLICATIONS.

THE MODERN PACKING HOUSE: A COMPLETE TREATISE ON THE DESIGNING, CONSTRUCTION, EQUIPMENT AND OPERATION OF A MODERN ABATTOIR AND PACKING HOUSE, ACCORDING TO PRESENT AMERICAN PRACTICE, INCLUDING FORMULAS FOR THE MANUFACTURE OF LARD AND SAUSAGE, THE CURING OF MEATS, ETC., AND METHODS OF CONVERTING ALL BY-PRODUCTS INTO COMMERCIAL ARTICLES. By F. W. Wilder. Chicago: Nickerson & Collins Co. 1905. 581 pp. Cloth, $10.00. Mor., $12.00.

L'Oxygène industriel et ses Applications. By R. Pictet. Paris: Fischbacher. 64 pp. 2 fr.

Introduction à Étude de la Chimie. By M. de Thierry. Paris: Masson et Cie. 14+456 pp. 10 fr.

Die Arzneimittel-Synthese auf Grundlage der Beziehungen zwischen chemischen Aufbau und Wirkung. Für Ärzte und Chemiker. By Sigmund Fränkel. Second edition. Berlin: J. Springer. 1906. 20+761 pp. Marks 16.

Leitfaden für die qualitative chemische Analyse. By Städeler-Kolbe. Neu-bearb. von Prof. Dr. H. Abeljanz. Edition 13. Zürich: Art. Institut Orelli Füssli. 1906. 13+128 pp. Marks 3.

Studien-Behelfe für den Unterricht in der allgemeinen und technischen Chemie. By Nicolas Teclu. Wien: (Leipzig: J. A. Barth). 1905. 5+270 pp. Marks 4.20.

Étude Comparative des Methodes d'Analyse des Engrais dans les Divers Pays. By L. Sicard. Montpellier: Coulet et fils. 2 fr. 50.

De l'Emploi des Alcaloides en Solution Huileuse. By Dr. Scrini. Paris: Vignt freres. 2 fr. 50.

Principles and Practice of Butter-Making: a Treatise on the Chemical and Physical Properties of Milk and its Components: the Handling of Milk and Cream and the Manufacture of Butter Therefrom. By G. L. McKay and C. Larsen. New York: J. Wiley & Sons. 1906. 12+329 pp. $1.50 net.

A Systematic Course of Qualitative Chemical Analysis of Inorganic and Organic Substances with Explanatory Notes. By H. W. Schimpf. New York: J. Wiley & Sons. 1906. 7+156 pp. $1.25 net.

The Conductivity of Liquids: Methods, Results, Chemical Applications and Theoretical Considerations. By Olin Freeman Tower. Easton, Pa.: Chemical Publishing Co. 1905. 4+182 pp. $1.75.

Elements of Quantitative Analysis. By G. H. Bailey. New York: Macmillan. 1905. 10+246 pp. $1.00.

Portland Cement, Its Manufacture, Testing and Use. By D. B. Butler. Second edition, revised and enlarged. New York: Spon & Chamberlain. 1906. 406 pp. $5.00 net.

Practical Cement Testing. By W. Purves Taylor. New York: Myron C. Clark Publishing Co. 1906. 8+320 pp. $3.00.

Experimental Electrochemistry. By Nevil Monroe Hopkins. New York: D. Van Nostrand Co. 1905. 14+284 pp. $3.00.

Special Experiments and Discussions in Introductory Chemistry: with a Plan for the Organization of the Subject-Matter. By Eugene Paul Schoch. Boston: D. C. Heath & Co. 1905. 7+62 pp. 50 cents.

KALENDER FÜR DIE GUMMI-INDUSTRIE UND VERWANDTE BETRIEBE 1906. Hrsg. von Edg. Herbst. Dresden: Steinkopff & Springer. 7+367 pp. geb. Marks 4.50.

KRITISCHE BETRACHTUNG EINIGER UNTERSUCHUNGSMETHODEN DER KAOLINE UND TONE. By Carl Loeser. Halle: L. Nebert. 1905. 29 pp. Mark 1.

L'APPAREILLAGE MÉCANIQUE DES INDUSTRIES CHIMIQUES. By A. Parnicke. Paris: H. Dunod. 356 pp. 12 fr. 50.

LA DISTILLATION DES RÉSINES ET LES PRODUITS QUI EN DÉRIVENT. By V. Schweizer. Paris: H. Dunod. 242 pp. 7 fr. 50.

DAIRY CHEMISTRY. By Harry Snyder. New York: The Macmillan Co. 1906. 11+190 pp. 90 cents.

CHEMICAL ANALYSIS, QUALITATIVE AND QUANTITATIVE. By W. Briggs and R. W. Stewart. Fifth impr. (Fourth edition.) Rev. and enl. by H. W. Bausor. London: Clive. 1906. 212 pp. 3/6.

MANUAL OF PHARMACOLOGY. By Walter E. Dixon. London: E. Arnold. New York: Longmans, Green & Co. 1906. 12+451 pp. $4.00.

A TREATISE ON PHARMACY FOR STUDENTS AND PHARMACISTS. By C. Caspari, Jr. Philadelphia: Lea Brothers & Co. 1906. 17+834 pp. $4.25.

URIC ACID: THE CHEMISTRY, PHYSIOLOGY AND PATHOLOGY OF URIC ACID. By Francis H. McCrudden. New York: Paul B. Hoeber. 1906. 11+318 pp. $2.50.

PRODUCER GAS AND GAS PRODUCERS. (A Treatise on the Subject of Gaseous Fluids.) By S. S. Wyer. New York: Engineering and Mining Journal. 1906. 300 pp. $4.00.

SECOND STAGE PRACTICAL INORGANIC CHEMISTRY. By W. Briggs and R. W. Stewart. Second edition. London: Clive. 1906. 172 pp. 2/.

SUGAR AND THE SUGAR CANE. (Elementary Treatise on the Agriculture of the Sugar Cane and on Manufacture of Cane Sugar.) By Nöel Deerr. London: Simpkin. 1906. 424 pp. 7/6.

CHEMISTRY SIMPLIFIED. By G. A. Koenig. Philadelphia: H. C. Baird. London: W. Dawson. 1906. 11/6.

CHEMISTRY OF THE PROTEIDS. By G. Mann. Based on Prof. Otto Cownheim's "Chemie der Eiweisskörper." London: The Macmillan Co. 1906. 624 pp. 15/.

SCIENCE CHEMISTRY PAPERS. (Questions set at Intermediate Science Examination of the University of London from 1882 to 1905.) London: Clive. 1906. 64 pp. 2/6.

EXPERIMENTAL ELECTROCHEMISTRY. By N. M. Hopkins. London: Constable. 1906. 298 pp. 12/.

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